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GENETIC ANALYSIS OF OBSTRUCTIVE SLEEP APNOEA AND ITS ASSOCIATIONS TO CARDIOMETABOLIC DISEASES AND COVID-19

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Doctoral Programme in Population Health

DOCTORAL DISSERTATION

To be presented for public discussion with the permission of
the Faculty of Medicine of the University of Helsinki, in Lecture hall 2,
Biomedicum, on 29th of October 2021, at 13 o'clock.

Helsinki 2021

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The cover image is adapted from the manuscript Strausz et al. (2020): *"Genetic analysis of obstructive sleep apnoea discovers a strong association with cardiometabolic health".*

Dissertationes Scholae Doctoralis Ad Sanitatem Investigandam Universitatis Helsinkiensis

ISBN 978-951-51-7442-0 (Print)
ISBN 978-951-51-7443-7 (Online)
ISSN 2342-3161 (Print)
ISSN 2342-317X (Online)

Unigrafia
Helsinki, 2021

To my lovely sons, Samuel and Joona

ABSTRACT

Obstructive sleep apnoea (OSA) is the most common sleep-related breathing disorder and is characterized by recurrent episodes of complete or partial obstruction of the upper airway leading to reduced or absent breathing during sleep. The prevalence of OSA in adults is approximately 25% in developed countries. The main known risk factors for OSA are increasing age, male sex, menopause, obesity and certain craniofacial structures and anomalies. The role of OSA on the risk of adverse cardiovascular outcomes has been widely studied, and mechanisms linking OSA to its cardiometabolic correlates through intermittent hypoxia, oxidative stress and increasing sympathetic activity are also recognized. Despite the fact that the epidemiology of OSA has been under research for decades, the genetics behind OSA risk have remained mainly unstudied. However, family studies have shown that family members are at a 2–4-fold greater risk of having OSA if there are OSA patients in the family. It is estimated that 40% of the variation in the apnoea-hypopnoea-index (AHI) is genetically regulated. Previous genome-wide-association studies (GWASes) have addressed OSA severity based on AHI or respiratory event duration, but case-control studies have not been previously published.

The World Health Organization (WHO) announced COVID-19 as a pandemic in March 2020. Patients with COVID-19 have a wide range of symptoms ranging from mild flu-like symptoms to severe illness. The first studies regarding COVID-19 revealed that male gender, higher age, obesity and diabetes are risk factors for the severe form of the disease, indicating that OSA and COVID-19 share numerous common risk factors and comorbidities. Furthermore, studies have suggested that OSA is a risk factor for the severe form of COVID-19.

We estimated the role of OSA in major cardiometabolic disease by utilizing population-based cohorts, including FINRISK, Health 2000 and a subset of the Botnia Study, and registry information to longitudinally assess OSA risk in the Finnish population. Our data consisted of 36,963 individuals with over 500,000 person-years and up to twenty-five years of follow-up data, including 1,568 OSA patients. Using Cox-proportional hazards models, our results revealed that OSA is associated with a 1.36-fold increased risk for coronary heart disease (CHD), including a 2.01-fold increased risk in women independent of other potential confounding factors. Similarly, type 2 diabetes (T2D) correlated with OSA independent of obesity status and revealed a 1.48-fold increased risk. This association was also significant in women, showing a 1.63-fold increased risk.

The risk of diabetic kidney disease (DKD) was increased by 1.75-fold in patients with OSA among the T2D study sample. All-cause mortality was increased in individuals with both OSA and T2D by 35%.

To study the genetic burden for the risk of OSA we proceeded to identify genetic loci associated with OSA risk and aimed to test if OSA and its comorbidities share a common genetic basis. To elucidate these aims, data from the FinnGen project was used. The FinnGen project combines patient genotype data and nationwide registry information with anthropometric measurements, such as body mass index (BMI) and smoking. Using this information, we conducted the first large-scale case-control GWAS of OSA with 217,955 individuals including 16,761 OSA patients. We identified five genetic loci associated with OSA, highlighting the importance of genetic variation on OSA predisposition. This was further supported by our single nucleotide polymorphism (SNP)-based heritability estimates. We also showed the causal relationship between obesity and OSA by utilizing Mendelian randomization (MR). Although BMI is the major risk factor, we were also able to find a BMI-independent genetic locus for OSA that is associated with antidepressant purchases. However, we could not replicate this locus in independent cohorts. In addition, we found strong genetic correlations between OSA and its comorbidities including BMI, hypertension, T2D, CHD, stroke, depression, hypothyroidism, asthma and inflammatory rheumatic diseases (IRD) in addition to other sleep traits such as sleepiness and sleep efficiency. These findings implicate OSA as a heterogenic disease with several distinct comorbidities, which would be beneficial to consider when treating patients with OSA.

When COVID-19 emerged, it became apparent that the risk factors for the severe form of the disease showed similarities with OSA risk factors and comorbidities. Our aim was to study if OSA patients have a higher risk for hospitalisation due to COVID-19 disease in addition to other potential confounding factors, and if OSA associates with an increased risk of contracting COVID-19. We studied 445 individuals with COVID-19 including thirty-eight OSA patients extracted from the FinnGen project data (N=260,405). Of the OSA patients, nineteen required hospital treatment due to COVID-19 infection. OSA was associated with a 2.93-fold increased risk of COVID-19 hospitalisation independent of age, sex, BMI and other comorbidities. The results were further confirmed in a meta-analysis including 15,835 individuals. Importantly, treatment information regarding OSA was also collected and suggested that moderate and severe OSA is a risk factor for severe COVID-19 even if the OSA is well managed.

This thesis concentrates on studying OSA as a risk factor for cardiometabolic comorbidities, the genetic variation between OSA and non-OSA individuals and whether OSA creates an elevated risk for severe COVID-19 disease. These studies were conducted by utilizing large and accurate data sets with an epidemiological and longitudinal ascertainment, and by applying modern genetic methods to show that OSA is a relevant topic during the exceptional times of the global COVID-19 pandemic.

TIIVISTELMÄ

Obstruktiivisella uniapnealla (uniapnea) tarkoitetaan toistuvia unenaikaisia vähintään kymmenen sekunnin mittaisia hengityskatkoksia (apnea) tai hengityksen vaimentumia (hypopnea), jotka johtuvat ylähengitysteiden ahtautumisesta. Uniapnean prevalenssin on arvioitu olevan jopa 25 % aikuisväestössä. Sen tunnetuimmat riskitekijät ovat korkea ikä, miessukupuoli, vaihdevuosi-ikä, ylipaino ja tietyt kraniofakiaaliset piirteet, kuten pieni tai takana sijaitseva alaleuka. Uniapnean ja sydän- ja verisuonitautien yhteyttä on tutkittu laajasti epidemiologisin tutkimusmenetelmin, ja niitä yhdistävät mekanismit, kuten jaksottainen hapenpuute, oksidatiivinen stressi ja uniapnean vaikutukset sympaattisen hermoston aktiivisuuden lisääntymiseen, tunnetaan. Epidemiologisista tutkimuksista ja tuloksista huolimatta uniapean genetiikan tutkimus on ollut melko vähäistä. Viitteitä on saatu siitä, että uniapneapotilaan perheenjäsenellä on 2–4-kertainen riski sairastua uniapneaan ja lisäksi on arvioitu, että 40 % apnea-hypopnea-indeksin variaatiosta on geneettisesti säädelty. Genominlaajuinen assosiaatioanalyysi on tuonut uuden menetelmän analysoida yleisiä kompleksisia tauteja, kuten uniapneaa. Näissä aiemmissa uniapneaa koskevissa tutkimuksissa on tutkittu taudin vaikeusastetta ja hengitystapahtuman kestoa. Kuitenkaan tapaus-verrokki -tutkimusta koskien uniapneaa ei ole aiemmin julkaistu.

Joulukuussa 2019 Kiinan Wuhanista alkoi epidemia, jonka aiheuttajana on ihmiselle uusi koronavirus. Sen aiheuttama tauti on viralliselta nimeltään COVID-19. Virus levisi nopeasti maailmanlaajuisesti ja maaliskuussa 2020 Maailman terveysjärjestö WHO julisti koronavirusepidemian pandemiaksi. COVID-19 aiheuttaa useimmille lieväoireisen hengitystieinfektion, mutta osalle potilaista infektio voi olla jopa henkeä uhkaava. Vakavan COVID-19 infektion riskitekijöihin kuuluvat mm. korkea ikä, ylipaino ja diabetes. Näin ollen COVID-19 ja uniapnea jakavat suuren määrän samoja riskitekijöitä ja liitännäissairauksia. Lisäksi on havaittu, että uniapnea voi lisätä riskiä vakalle COVID-19-infektiolle.

Arvioimme uniapnean aiheuttamaa riskiä koronaaritaudille, tyypin 2 diabetekselle, diabeteskomplikaatioille ja kuolleisuudelle hyödyntämällä FINRISKI ja Terveys 2000 kohortteja sekä osajoukkoa Botnia-tutkimuksesta. Tutkimuksemme sisälsi 36 963 henkilöä ja se kattoi yli 500 000 henkilövuotta yltäen jopa yli 25-vuoden seuranta-aikaan. Käyttämällä Cox-elinaikamallia havaitsimme, että uniapnea liittyy koronaaritaudin riskiin 1.36-kertaisesti riippumatta muista riskitekijöistä, kuten verenpainetaudista tai painoindeksistä. Tämä yhteys nähtiin myös naisilla, joilla yhteys riskiin nousi 2.01-kertaiseksi. Vastaavasti uniapnean havaittiin olevan ylipainosta riippumaton riskitekijä tyypin 2 diabetekselle liittyen riskiin 1.48-kertaisesti. Vaikutus havaittiin myös naisilla, joilla uniapnea yhdistyi 1.63-kertaiseen riskiin. Lisäksi uniapnea assosioitui kohonneeseen riskiin diabeetikkojen munuaissairauksille 1.75-kertaisesti ja uniapnean nähtiin lisäävän kuolleisuutta 35 % tyypin 2 diabeetikoilla.

Tutkiaksemme uniapnean genetiikkaa hyödynsimme FinnGen-aineistoa, joka yhdistää genomitietoja kansallisiin terveysrekistereihin. Tarkastelimme uniapneaa käyttämällä genominlaajuista assosiaatioanalyysiä sisältäen 217 955 henkilöä, joista 16 761:lla oli uniapneadiagnoosi. Löysimme viisi uniapneariskiin assosioituvaa lokusta. Tämä tutkimus korostaa geneettisen variaation merkitystä uniapnealle altistumisessa, jota heritabiliteettilöydöksemme vahvistaa. Näytimme lisäksi mendeliaanisen randomisaation avulla, että ylipainon ja uniapnean välillä on kausaalinen suhde, joka on ollut nähtävissä epidemiologisissa tutkimuksissa. Huolimatta siitä, että ylipaino on uniapnean tärkein riskitekijä, löysimme painoindeksistä riippumattoman lokuksen, joka oli spesifi uniapneadiagnoosille, ja joka yhdistyi masennuslääkeostoihin. Tätä tulosta emme kuitenkaan onnistuneet toistamaan itsenäisissä aineistoissa. Havaitsimme voimakkaita geneettisiä korrelaatioita uniapnean ja sen liitännäissairauksien, kuten verenpainetaudin, tyypin 2 diabeteksen, koronaaritaudin, depression, kilpirauhasen vajaatoiminnan, astman ja inflammatoristen reumasairauksien välillä. Lisäksi geneettiset korrelaatiot olivat vahvoja uniapean ja päiväväsymyksen sekä unen tehokkuuden välillä. Nämä havainnot viittaavat siihen, että uniapnea on heterogeeninen sairaus, johon liittyy useita eri tauteja, jotka tulisi huomioida uniapneapotilaita hoidettaessa.

Tarkastelimme lisäksi uniapnean aiheauttamaa riskiä vakavalle COVID-19-infektiolle ja mahdollisesti uniapneapotilaiden suurentunutta riskiä saada infektio. Hyödyntämällä FinnGen-kohorttia (N=260 405) aineistomme sisälsi 445 COVID-19 positiivista henkilöä, joista 38:lla oli lisäksi uniapneadiagnoosi. Heistä 19 tarvitsi sairaalahoitoa COVID-19 aiheuttaman infektion vuoksi. Uniapnea liittyi 2.93-kertaiseen riskiin vakavalle infektiolle riippumatta muista riskitekijöistä, kuten iäistä, sukupuolesta, painoindeksistä, verenpainetaudista, tyypin 2 diabeteksesta, koronaaritaudista, keuhkoahtaumataudista ja astmasta. Lisäksi teimme meta-analyysin, joka sisälsi 15 835 COVID-19 positiiviseksi testattua henkilöä vahvistaen tietoutta uniapnean aiheuttamasta kohonneesta riskistä. Keräsimme uniapneapotilaiden hoitotietoja ennen sairastumista COVID-19-infektioon ja havaitsimme, että vaikka suurin osa uniapneapotilaista oli hoidettu, vaativat he silti sairaalahoitoa. Tämä antaa viitteitä siitä, että keskivaikea ja vaikea uniapnea on hoidettunakin riskitekijä vakavalle COVID-19-infektiolle.

Tämä väitöskirja tutkii uniapnean yhteyttä kardiometabolisten sairauksien ilmaantuvuuteen ja niiden riskitekijöihin. Samalla se selvittää geneettistä vaihtelua uniapneapotilaiden ja ei-uniapneapotilaiden välillä tarkastellen lisäksi uniapnean geneettistä yhteyttä sen liitännäissairauksiin hyödyntämällä laajoja kansallisia aineistoja epidemiologisesta ja geneettisestä näkökulmasta luoden katsauksen uniapneasta suomalaisessa väestössä. Tutkimuksissa on hyödynnetty niin pitkittäistutkimukseen soveltuvia kuin uusimpia geneettiseen laskentaan kehiteltyjä menetelmiä. Lisäksi keskivaikean ja vaikean uniapnean yhteys COVID-19-infetion vakavaan muotoon nostaa sen hyvin ajankohtaiseksi aiheeksi pandemian aikana.

CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications:

I. Strausz S, Havulinna AS, Tuomi T, Bachour A, Groop L, Mäkitie A, Koskinen S, Salomaa V, Palotie A, Ripatti S, Palotie T. Obstructive sleep apnoea and the risk for coronary heart disease and type 2 diabetes: a longitudinal population-based study in Finland. BMJ Open. 2018 Oct 15;8(10).

II. Strausz S, Ruotsalainen S, Ollila HM, Karjalainen J, Kiiskinen T, Reeve M, Kurki M, Mars N, Havulinna AS, Luonsi E, Mansour Aly D, Ahlqvist E, Teder-Laving M, Palta P, Groop L, Mägi R, Mäkitie A, Salomaa V, Bachour A, Tuomi T, Palotie A, Palotie T, Ripatti S; FinnGen research group. Genetic analysis of obstructive sleep apnoea discovers a strong association with cardiometabolic health. Eur Respir J. 2020 Dec 10:2003091.

III. Strausz S, Kiiskinen T, Broberg M, Ruotsalainen S, Koskela J, Bachour A; FinnGen, Palotie A, Palotie T, Ripatti S, Ollila HM. Sleep apnoea is a risk factor for severe COVID-19. BMJ Open Respir Res. 2021 Jan;8(1).

The original publications are referred to in the text by their roman numerals.

ABBREVIATIONS

A: Adenine
AHI: Apnoea-hypopnoea-index
ANDIS: The All New Diabetics in Scania
BMI: Body mass index
C: Cytosine
CAMK1D: Calcium/calmodulin-dependent protein kinase 1D
CHD: Coronary heart disease
CI: Confidence interval
COPD: Chronic obstructive pulmonary disease
CPAP: Continuous positive airway pressure
CS: Continuous shrinkage
CSA: Central sleep apnoea
CXCR4: C-X-C motif chemokine receptor 4
DKD: Diabetic kidney disease
DNA: Deoxyribonucleic acid
ENCODE: Encyclopaedia of DNA Elements
ESS: Epworth Sleepiness Scale
ESTBB: Estonian Biobank
FTO: Fat mass and obesity-associated protein
G: Guanine
GAPVD1: GTPase activating protein and VPS9 domains 1
GATK: Genomic analysis toolkit
GTEx: Genotype-Tissue Expression
GWAS: Genome-wide association study
H2000: Health 2000 Survey
HDL: High density lipoprotein
HR: Hazard ratio
HUH: Helsinki University Hospital
HUS: Hospital District of Helsinki and Uusimaa
ICD: International Classification of Diseases
INFO: Imputation quality score
IQR: Interquartile range
IRD: Inflammatory rheumatic diseases
LD: Linkage disequilibrium
LDSC: Linkage disequilibrium score regression
MAD: Mandibular advancement device
MAGMA: Multi-marker analysis of genomic annotation

MR: Mendelian randomization
NEDD1: NEDD1 gamma-tubulin ring complex targeting factor
OCST: Out-of-centre sleep study
ODI: Oxygen desaturation index
OR: Odds ratio
OSA: Obstructive sleep apnoea
PC: Principal component
PCR: Polymerase chain reaction
PheWAS: Phenome-wide association study
PPV: Positive predictive value
PRS: Polygenic risk score
PSG: Polysomnography
RDI: Respiratory disturbance index
REI: Respiratory event index
REML: Restricted maximum likelihood method
RFA: Radiofrequency ablation
RMST: Rhabdomyosarcoma 2 associated transcript
SAIGE: Scalable and accurate implementation of generalized mixed model
SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2
SD: Standard deviation
SISu: Sequencing Initiative Suomi
SNP: Single nucleotide polymorphism
T: Thymine
T1D: Type 1 diabetes
T2D: Type 2 diabetes
THL: The Finnish Institute for Health and Welfare
UKBB: UK Biobank
UPPP: Uvulopalatopharyngoplasty
WHO: World Health Organisation

1 INTRODUCTION

Sleep apnoea is a common sleep-related breathing disorder causing reduced or absent breathing during sleep. Sleep apnoea can be divided into three groups: obstructive sleep apnoea (OSA), central sleep apnoea (CSA) and mixed sleep apnoea, which is a combination of both OSA and CSA. OSA represents the majority of cases of sleep apnoea (1, 2) and is a widely prevalent disease especially in developed countries with an estimated prevalence of approximately 25% among the general population (1). The prevalence of OSA is highly age dependent, increasing up to 40% among individuals aged 50 to 70 years (3).

The main risk factors for OSA include older age, male sex, menopause, obesity and craniofacial structure variations and anomalies (4). OSA has been established as a risk factor for cardiovascular diseases through sympathetic activation, oxidative stress, and intermittent hypoxia (5-7).

Genetic factors contribute to the main risk factors for OSA, such as obesity and craniofacial structures (8, 9). However, the genetic association to OSA itself has not been adequately studied, although for example family members are estimated to have a 2–4-fold greater risk of having OSA, indicating a genetic predisposition (10). Therefore, the genetic risk factors for OSA remain an important subject of investigation.

Genome-wide association studies (GWASes) concerning OSA severity and respiratory event duration exist (11-13), but GWAS on apnoea risk comparing apnoea patients and healthy controls have not been published until now. Biobanks and longitudinally-collected registry-based data have created a unique environment to investigate common diseases, such OSA, over a patient's lifespan. The FinnGen biobank project combines genome and registry data with anthropometric measurements such as body mass index (BMI) and smoking, providing an excellent resource for the study of common complex diseases such as OSA.

COVID-19, which is caused by the virus severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was first identified in December 2019 in Wuhan, China. SARS-CoV-2 quickly spread worldwide, and the new virus was declared a pandemic in March 2020 by the World Health Organisation (WHO) (14). The risk of developing a severe COVID-19 illness is increased by pre-existing conditions that significantly impair the functioning of the lungs, heart, or immune system (15). Severe COVID-19 and OSA also share multiple common risk factors and comorbidities, such as higher age, obesity, and diabetes. Furthermore, studies suggest that OSA is a risk factor for severe COVID-19 illness (16-21).

This thesis investigates the connection between OSA and the incidence of cardiometabolic diseases and their risk factors, how genetic variation influences the risk for OSA and whether there is an increased risk for severe illness when contracting novel respiratory viruses among OSA patients.

2 REVIEW OF THE LITERATURE

2.1 Sleep apnoea

Sleep apnoea is a common sleep-related breathing disorder causing reduced or absent breathing during sleep. Sleep apnoea is traditionally divided into three categories: OSA, CSA and mixed sleep apnoea, which is a combination of both OSA and CSA. OSA is clearly the most common of these forms (1, 2).

CSA is defined by a lack of respiratory effort during cessations of airflow while asleep, in contrast to OSA where respiratory efforts are observed during the respiratory events. Several manifestations of CSA exist including idiopathic CSA, obesity hypoventilation syndrome, narcotic-induced CSA, and high altitude-induced periodic breathing. The most important of the CSA manifestations is Cheyne-Stokes breathing, which is typically seen in medical disorders such as heart failure, cerebrovascular disorders, and renal failure (22). The prevalence of CSA strongly depends on its form and it is overall rare, affecting approximately 1% of individuals (2). CSA is considered the primary diagnosis when ≥ 50% of apnoeas are scored as central in origin (22).

Mixed apnoea is a combination of OSA and CSA. It is typically characterized by absence of respiratory effort and airflow in the first section of the event, such as occurs in CSA, and respiratory effort without airflow in the last section, as it occurs in OSA (23).

2.1.1 Overview of obstructive sleep apnoea

OSA is a disorder of repetitive pharyngeal collapse during sleep, where the pharyngeal airway lumen size is decreased by craniofacial structures or body fat (24). This could lead to an elevated probability of pharyngeal collapse in the majority of the OSA cases (25). Although this consequence is often seen in OSA, several other factors also contribute, including poor upper airway muscle function, low arousal threshold and low lung volume (26). Pharyngeal collapse can present as a complete collapse causing apnoea, or partial collapse causing hypopnoea. The airway is held open through the activity of upper airway dilator muscles which are highly active during wakefulness. During sleep the muscle activity is reduced, especially during deep sleep, and the airway collapses (27). Most often the collapse occurs at the level of oropharynx, with the extension to laryngopharynx (also referred as hypopharynx) is commonly observed (28), (Figure 1).

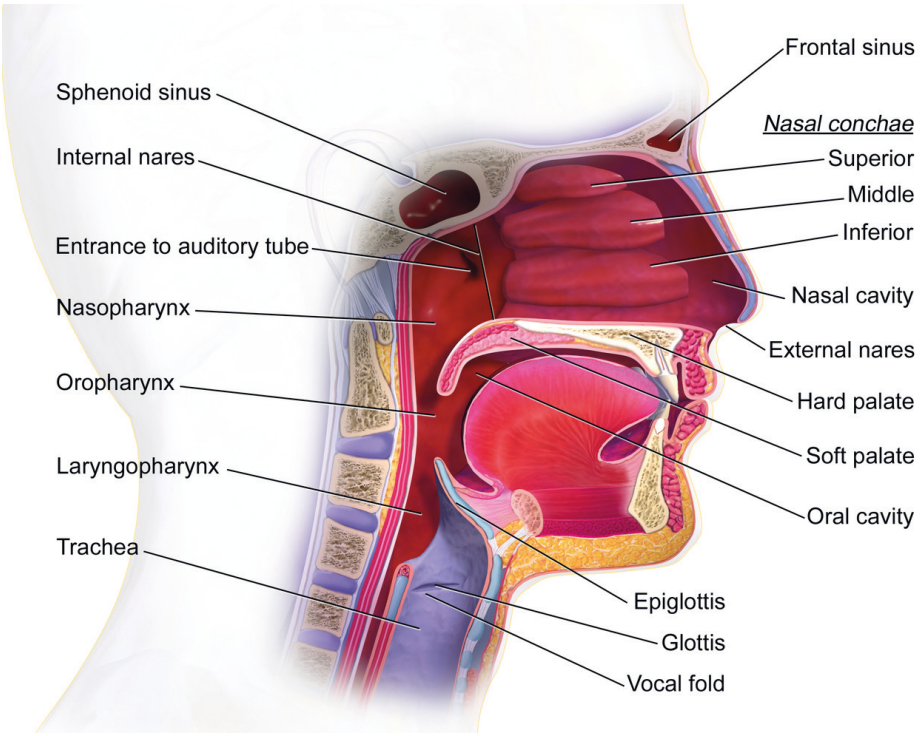

Figure 1. The upper respiratory system. In obstructive sleep apnoea, the pharyngeal collapse occurs most often at the level of oropharynx, with the extension to laryngopharynx commonly observed. This figure is adapted from Blausen.com staff (2014); "Medical gallery of Blausen Medical 2014" under the Creative Commons Attribution License.

Pharyngeal collapses cause pauses in breathing which lead to acute adverse effects, including sleep fragmentation, fluctuations in blood pressure and heart rate, increased sympathetic activity, cortical arousal and oxyhaemoglobin desaturation. These adverse effects result in OSA causing cardiometabolic and neurocognitive consequences over time (4).

2.1.1.1 Prevalence

OSA prevalence in the general adult population is approximately 25% (1). An estimated 29.5% of Finnish individuals aged 30-69 have OSA (29). Concerningly, in the Wisconsin Sleep Cohort Study, 34% of men and 17% of women aged 30 to 70 years had at least mild OSA with a 2-fold increased risk for men compared to women. The prevalence of OSA is tightly age dependent and it is increased among advanced age groups, with a prevalence of 43% in men and 28% in women among patients aged 50 to 70 years. OSA prevalence has increased approximately 30% between 1990 and 2010, along with population BMI, the most important risk factor for OSA (3).

2.1.1.2 Diagnosis

Diagnosing OSA is based on a comprehensive sleep evaluation where clinical signs and symptoms are measured. This consists of anamnestic information including a sleep-oriented history, physical examination, and findings from sleep testing. The diagnosis is always verified by sleep testing followed by the interpretation of a sleep specialist (30).

2.1.1.2.1 Anamnesis and physical examination

The examination for OSA starts with an anamnesis including assessment of sleep history, which is typically obtained as a part of a routine health maintenance evaluation in a primary health care setting (30). Sleep history may be initially assessed by a questionnaire (e.g., Basic Nordic Sleep Questionnaire (31)) or the following questions can be used to screen whether a patient has a sleep disorder that requires clinical attention: 1) Does the patient suffer from difficulty falling asleep or daytime fatigue at least three days a week, 2) Does the sleep disorder impair the patient's ability to function during the day. If the answer is positive to at least one of the questions, it is appropriate to proceed with a more detailed examination (32).

In the examination, the presence of obesity and OSA-predisposing craniofacial structures such as retrognathic jaws, tonsillar and adenoidal hypertrophy and increased neck circumference should be evaluated (30). Additionally, information concerning other diseases, medication, smoking status, alcohol consumption and shift work should be evaluated. OSA-related symptoms should also be assessed (see Table 1) (33-35). Positive findings on this OSA screen should lead to a more comprehensive evaluation of sleep history and physical examination (30).

Table 1. Signs and symptoms suggestive of obstructive sleep apnoea (OSA)

Symptoms of OSA	
Symptoms while awake	**Symptoms while asleep**
Excessive daytime sleepiness	Snoring
Morning headaches	Respiratory interruptions
Tendency to doze off	Restless sleep
Memory impairment	Night time perspiration
Difficulty concentrating	Increase need to urinate during the night
Mood changes	Heartburn
Impotence, impaired libido	Dry mouth
Coughing	Drooling
	Insomnia

The most common day and night-time symptoms associated with OSA according to previous studies (33-35).

When a patient is suspected of having OSA, a comprehensive sleep history should include an evaluation for snoring, witnessed apnoeas, gasping/choking episodes, total sleep amount, nocturia, morning headaches, sleep fragmentation/ sleep maintenance insomnia, decreased concentration and memory, and excessive sleepiness not explained by other factors (30). The Epworth Sleepiness Scale (ESS) is used to assess the severity of sleepiness. The ESS is an eight-question questionnaire which measures an individual's general level of daytime sleepiness, or their average sleep propensity in daily life (36). Secondary conditions that may be caused by OSA, including hypertension, myocardial infarction, pulmonary heart disease, diabetes, stroke, decreased daytime alertness, depression and also medication and a history of motor vehicle accidents, should be screened (30).

An increased risk of OSA can be recognised through a physical examination and therefore an inspection of respiratory, cardiovascular, and neurologic systems should be carried out (37). Features to be evaluated that may suggest the presence of OSA include a BMI ≥30 kg/m^2, increased neck circumference (>43 cm in men, > 41cm in women), a score of 3 or 4 on the modified Mallampati scale (originally created to predict difficult tracheal intubation, but which can be used in general to estimate narrow airways (38, 39)) and certain characteristics of craniofacial structures such as the presence of retrognathia and/or opening growth direction of the lower jaw, macroglossia and nasal abnormalities (40, 41).

Following anamnesis and physical examination, patients can be classified according to their risk for OSA. The high-risk category includes obese patients or those with cardiometabolic comorbidities such as treatment-resistant hypertension, type 2 diabetes (T2D), heart failure, atrial fibrillation, stroke, nocturnal dysrhythmias, pulmonary hypertension and professional drivers or pilots. High risk patients should be prioritised to have their diagnoses and disease severity assessed by out-of-centre sleep study (OCST) or polysomnography (PSG) testing (30).

2.1.1.2.2 Sleep testing

A diagnosis of OSA must always be established through in-laboratory or ambulatory PSG or OCST (30).

Overnight PSG in a laboratory is the gold standard for diagnosing OSA, with the primary outcome measure being the apnoea-hypopnoea-index (AHI). The definitions for apnoea, hypopnoea and the measurements mainly used to assess these are outlined in Table 2. PSG includes concurrent screening of both sleep and respiration. To follow the sleep–wake state, electroencephalogram, left and right electrooculogram, and chin electromyogram are documented. The recording of respiration includes airflow monitoring, respiratory effort measurement and oxygen saturation. Additional recommended parameters include body position and leg electromyography derivations (30).

Table 2. Definitions for the main measurements concerning obstructive sleep apnoea

Measure	Definition
Apnoea	Apnoea is scored when there is a drop in the peak signal excursion by ≥ 90% of pre-event baseline for ≥ 10 seconds.
Hypopnoea	Hypopnoea is scored when the peak signal excursions drop by ≥ 30% of pre-event baseline for ≥ 10 seconds in association with either ≥ 3% arterial oxygen desaturation or an arousal.
AHI: Apnoea-hypopnoea-index	The average number of apnoeas and hypopnoeas per hour of sleep.
REI: Respiratory event index	The average number of apnoeas and hypopnoeas per hour of sleep, measured during out-of-centre sleep study.
ODI_3 or ODI_4: Oxygen desaturation index	The oxygen desaturation index is the number of times per hour of sleep that the blood's oxygen level drop 3 or 4 percent from the baseline.

The definitions are based the American Academy of Sleep Medicine (AASM) published rules for scoring respiratory events in the *AASM Manual for the Scoring of Sleep and Associated Events* (23).

As the diagnosis of OSA is defined by PSG, the protocol is time consuming and expensive. OCST has been evaluated to have 80% specificity when compared to PSG and thus it has been increasingly investigated as a diagnostic alternative to PSG (42). However, if the finding of OCST is mild or moderate, but the patient appears to have severe OSA by clinical assessment, the examination should be re-done with PSG or OCST or the patient should have a treatment trial with a self-adjusting continuous positive airway pressure (CPAP) appliance (33). OCST may underestimate AHI and thus a fraction of OSA patients may remain undiagnosed when OCST is used (43).

A diagnosis of OSA is confirmed if AHI on PSG or respiratory event index (REI) on OCST is ≥15 events per hour or ≥5 per hour in addition to either signs or symptoms for OSA (e.g., snoring, fatigue, associated sleepiness, insomnia, nocturnal respiratory disturbance, or observed apnoea) or associated medical or psychiatric disorder (i.e., hypertension, diabetes, coronary heart disease (CHD), heart failure, atrial fibrillation, stroke, cognitive dysfunction, or mood disorder). OSA severity is defined as mild for AHI or REI ≥5 and <15, moderate for AHI or REI ≥15 and ≤30, and severe for AHI or REI >30 per hour (44).

2.1.1.2.3 Distribution of care between health care sectors

Diagnosis and treatment of OSA involve cooperation between primary and secondary health care sectors. In primary care, OCST can be ordered when OSA is suspected. Patients with high-risk occupations, such as pilots and professional drivers, and patients with moderate or severe OSA should be referred to a secondary

health care visit. Evaluation of CPAP treatment and OSA surgery takes place in the secondary health care sector (33).

2.1.1.3 Risk factors

Several predisposing factors for OSA are known, including obesity and certain craniofacial structures. In addition, male sex has been established as one of the main risk factors for OSA, as males have a three- to five-fold OSA risk compared to females (10, 41, 45-57). The most relevant risk factors and associated comorbidities are presented below and are outlined in Figure 2.

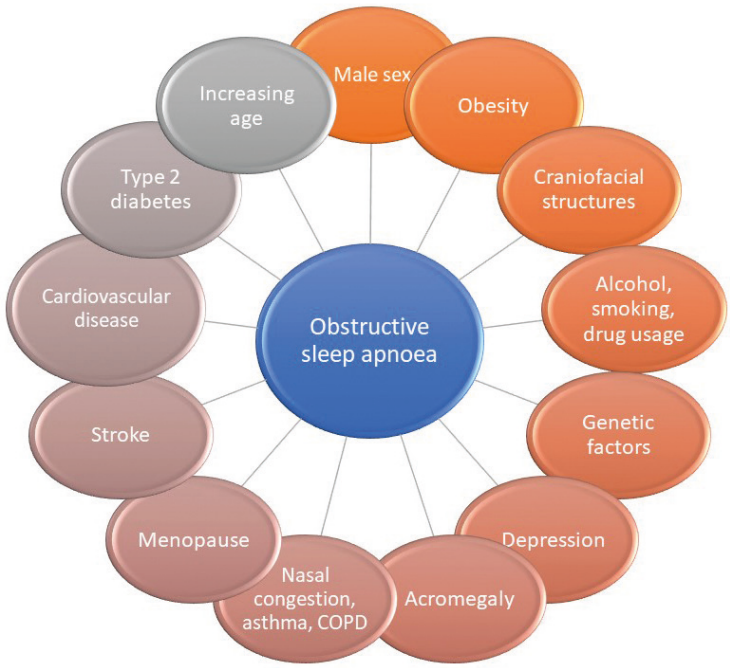

Figure 2. Predisposing factors for obstructive sleep apnoea (OSA) and its known comorbidities. Risk factors and comorbidities for OSA are gathered from previous studies concerning OSA (10, 41, 45-57).

2.1.1.3.1 Genetic factors

Genetic factors predisposing individuals to OSA are mainly unstudied, but important existing studies and findings are presented in the section: "7.2.2 Genetic studies concerning sleep apnoea".

2.1.1.3.2 Demographic factors

OSA has been considered mainly a male disease with male-female ratios (varying from 3:1 to 5:1) indicating that women with OSA are less likely to be diagnosed than men (58). Furthermore, OSA in women may be diagnosed later than in men

or may not be effectively treated (59, 60). Women have been shown to have higher impairment of quality of life and their healthcare disbursements have been higher compared to males with similar AHI values (61).

Increasing age is an established risk factor for OSA. The Wisconsin Sleep Cohort Study revealed in unadjusted estimates that 34% of men and 17% of women aged 30 to 70 years had at least mild OSA and the corresponding percentages were 43% and 28% among patients aged 50 to 70 years (3). In addition, it is estimated that individuals aged 65 or older have a 2- to 3-fold OSA risk compared to individuals aged 30-64 years (62, 63).

2.1.1.3.3 Obesity

Overweight and obesity are defined as abnormal or excessive fat accumulation that presents a risk to health. WHO has created a classification to assess weight by BMI, defining underweight as <18.5 kg/m^2, normal range as 18.5-24.9 kg/m^2, overweight as 25.0-29.9 kg/m^2 and obesity as ≥30 kg/m^2 (64).

Obesity is the most important risk factor for OSA. Two out of three OSA patients are overweight. Strong epidemiological evidence suggests that excess weight is a causal factor for the development of OSA (24). A 10% weight gain is associated with a 6-fold increased risk for OSA in individuals who have not had OSA at baseline and an approximately 32% increase in AHI for those with OSA. Comparably, among patients who have lost weight a significant improvement in OSA severity has been seen; a 10% decrease in weight has been associated with a 26% decrease in AHI (65).

2.1.1.3.4 Craniofacial structures

Craniofacial and upper-airway structures are important characteristics in the development of OSA (66). Influencing factors include skeletal or soft tissue variations and anomalies, which are often linked to convex facial profile, the presence of retrognathia and/or opening growth direction of the lower jaw, increased anterior lower facial height, high arched/narrowed hard palate, tonsillar and adenoidal hypertrophy, macroglossia, elongated/enlarged uvula, elongation of the soft palate, inferiorly positioned hyoid bone and narrowed nasal cavities. These features cause upper airway narrowing and thus predispose an individual to the development of OSA (40, 41). Among children, enlarged tonsils and adenoids might cause unfavourable growth rotations of the lower jaw and hence increase the risk for OSA (67).

2.1.1.3.5 Hormonal changes

In women, sex hormone levels are largely changing during menarche, pregnancy and menopause. Sex hormones regulate respiration both directly and through central neuromodulatory serotonergic neurons, essentially involved in the neural

control of breathing (68, 69). Thus, it is likely that sex hormone variation has an effect on the risk of developing OSA. It was shown that postmenopausal women had a 3-fold increased risk in comparison to premenopausal women to have moderate or severe OSA, independent of other potential confounding factors. This association was attenuated when comparing postmenopausal hormonal therapy users with premenopausal women (46). In addition, hormone therapy users 50 years or older compared with individuals who are not using hormonal therapy showed a significant 45% reduced risk for OSA (70). However, a randomized trial involving postmenopausal women revealed only a mild association with hormone therapy in reducing AHI (71).

OSA is one of the most common comorbidities among acromegaly patients, affecting 20% to 80% of individuals (48). Hypersecretion of growth hormone and insulin-like growth hormone leads to upper airway narrowing by pharyngeal soft tissue swelling and macroglossia (72, 73). OSA is also considered a clinical marker of acromegaly when defining the diagnosis (74). Whether acromegaly is treated with medication or surgery, AHI is significantly reduced (75).

2.1.1.3.6 Nasal congestion

Nasal congestion can be caused by anatomy, acute upper respiratory infection, or allergic rhinitis. All the above have been associated with snoring and OSA (45). OSA has been observed to worsen during the allergy season among patients who are suffering from seasonal allergic rhinitis. The risk for OSA has been shown to be increased 1.8-fold when comparing individuals with chronic nasal congestion to no nasal congestion (47).

2.1.1.3.7 Alcohol consumption, smoking and drug usage

Alcohol consumption has been seen to increase the risk of OSA 1.25-fold when comparing alcohol-users and non-alcohol users (76). Alcohol ingestion increases upper airway collapsibility and induces apnoeic episodes (77). Smoking also has detrimental effects on OSA associated with airway inflammation, nasopharyngeal oedema and smoking-related diseases (49, 78).

Medications and substances that relax muscles or suppress respiratory drive (e.g., benzodiazepines, and opioids) may also contribute to OSA risk and should be minimized or avoided (79).

2.1.1.4 Cardiometabolic comorbidities

2.1.1.4.1 Mechanisms

There are at least three potential mechanisms for how OSA interacts with its associated cardiometabolic diseases, such as T2D, hypertension, CHD and stroke (Figure 3). First, OSA causes intermittent hypoxia which leads to increased oxidative stress. This oxidative stress further causes systemic inflammation and increases sympathetic activity. Second, intrathoracic pressure changes lead to excessive mechanical stress on the heart and large arteries. Third, arousal-induced reflexive sympathetic activation results in repetitive increases in blood pressure (5).

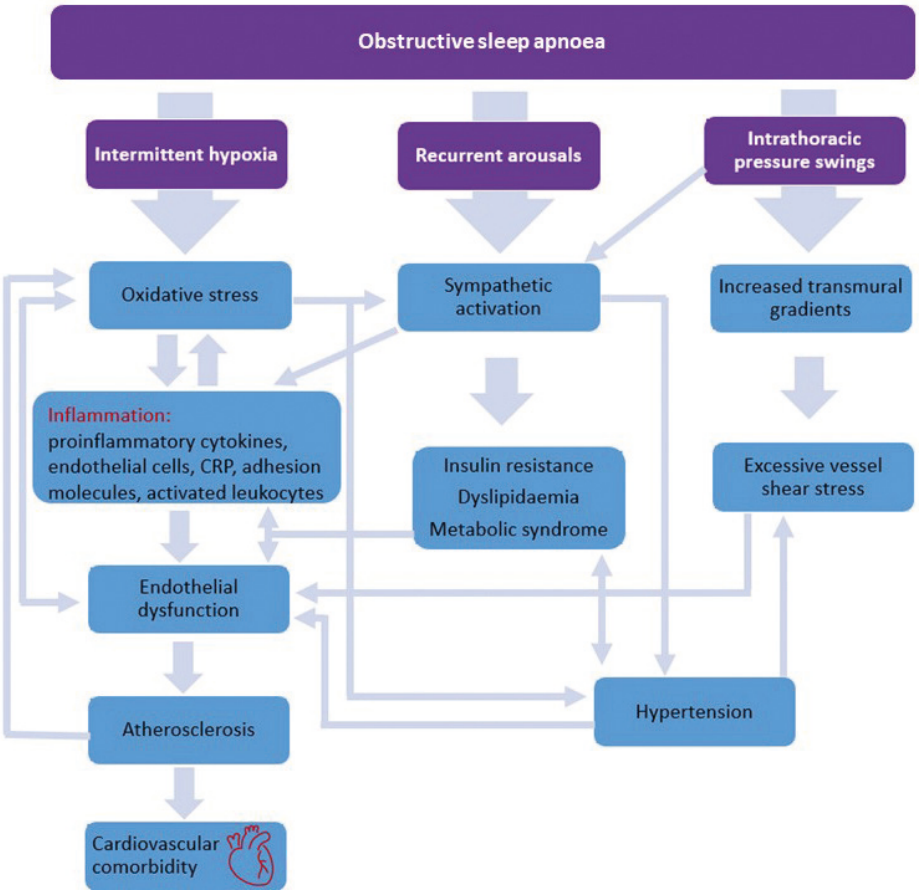

Figure 3. Potential mechanisms of the interactions between obstructive sleep apnoea and cardiometabolic diseases.

2.1.1.4.2 Cardiovascular risk factors

The prevalence of hypertension is linked with OSA severity ranging from 30 to 80% prevalence from mild to severe OSA (50). In addition, moderate to severe OSA is associated with a 3-fold increased risk for developing hypertension compared to individuals who do not have OSA (80). Each apnoeic event per hour increases the odds of hypertension by 1% (81).

Patients with hypertension are also diagnosed with OSA in approximately 40% of cases. OSA is even more common among patients with treatment resistant hypertension, as OSA is diagnosed in 83% of these patients (82). Importantly, the treatment of OSA is associated with a significant reduction in both systolic and diastolic blood pressure (83, 84) and a positive correlation has been shown between number of hours of CPAP use (especially in patients with at least 4 hours of use per night) and the decrease in blood pressure levels (85).

The association between OSA and plasma lipid levels has been under investigation. The prevalence of hyperlipidaemia varies from 15.1% in subjects without OSA to 26.1% in patients with OSA (86). As OSA and hyperlipidaemia are both strongly linked with cardiovascular diseases, there is also evidence that OSA severity associates independently with cholesterol and triglyceride concentrations. High density lipoprotein (HDL) in particular has been shown to be significantly reduced as AHI increased (87). Furthermore, CPAP treatment may improve dyslipidaemia by decreasing total cholesterol and low density lipoprotein and increasing HDL (88).

Current smoking is an established risk factor for cardiovascular disease, and the risk remains significantly elevated for at least 5 to 10 years after cessation relative to never smokers (89). Smoking has not only been shown to associate with cardiovascular disease, but also increases OSA severity (78).

2.1.1.4.3 Cardiovascular diseases

Based on epidemiological studies, OSA associates with many different forms of cardiovascular diseases such as CHD, atrial fibrillation, and stroke (6). OSA is highly prevalent as it is estimated to affect 40% to 60% of patients with cardiovascular disease (7).

In a prospective longitudinal epidemiological study, the Sleep Heart Health Study, OSA was found to be a significant predictor of CHD, increasing the risk by 10% per 10-unit increase in AHI after adjustment for multiple risk factors. Interestingly, this risk was observed in men aged 70 years or younger, but not in men over 70 years or in women (90). In addition to AHI, hypopneas with an oxyhaemoglobin desaturation of 4% or more associated independently with cardiovascular disease (91).

The association of OSA severity to cardiovascular outcomes has also been investigated. A meta-analysis consisting of 16 cohort studies and 24,208

individuals suggested that severe OSA is associated with increased CHD risk by 63%, moderate OSA by 38%, but no significant association was seen between mild OSA and CHD (92).

OSA is highly prevalent among stroke patients and the reported frequencies vary from 30% to 80% (93). Based on the aforementioned meta-analysis, severe OSA was associated with a 2.15-fold increased risk of stroke, but no association was observed between moderate or mild OSA and stroke (92). This finding is in line with a landmark study where an independent association between severe OSA and stroke was found (94).

2.1.1.4.4 Type 2 diabetes and its complications

Many comorbidities of OSA overlap with the comorbidities of T2D including obesity and hypertension. It has been suggested that OSA has a role in the progression of metabolic syndrome through the development of insulin resistance (5). The prevalence of OSA among T2D individuals is estimated to be between 58 to 86% (55, 95). Similarly, the unadjusted prevalence of T2D increases with OSA severity, with a 7% prevalence among OSA-free individuals and 29% among patients with severe OSA. Furthermore, after adjusting for obesity and other confounding factors, severe OSA was associated with an increased risk for T2D by 1.87-fold, moderate OSA 1.73-fold and mild OSA 1.33-fold (96).

OSA is also associated with T2D complications, such as kidney disease, indirectly through its adverse effects of hypertension, oxidative stress, and hypoxemia-related consequences (97). However, this association can be observed in both directions i.e., OSA may contribute to impaired renal function and impaired renal function may cause OSA. Therefore, OSA may also increase the risk for kidney dysfunction especially in the population of T2D individuals (98).

2.1.1.5 Other comorbidities

In addition, numerous other comorbidities have been reported to associate with OSA including depression, hypothyroidism, asthma and inflammatory rheumatic diseases (IRD) (99-103).

The prevalence of depression has been noted to be higher among OSA patients compared to OSA-free individuals and is related to OSA severity. The estimates of depression rates among OSA individuals vary from 17% to 41%. However, the connection between these two diseases is complex, as both depression and OSA share a number of similar symptoms such as fatigue and sleep problems (100).

Additionally, hypothyroidism and OSA have overlapping symptoms. Hypothyroidism alone does not usually cause OSA, but if other risk factors, such as obesity or male gender, are present these may create a risk for OSA (53). In addition, the contributing factors for OSA include low respiratory drive, upper airway narrowing by myxedema or obesity (53, 104). Newly diagnosed hypothyroidism

among OSA patients is not significant, but subclinical hypothyroidism is more prevalent in the OSA population (99).

In addition to OSA, chronic obstructive pulmonary disease (COPD) is a common respiratory disease affecting 14% of men and 4% of women (105). OSA prevalence varies among COPD patients from 10% up to 65.5% (57, 106). Patients with COPD and OSA are at an increased risk of death and exacerbations of COPD if OSA remains untreated (107, 108). Concurrence of these diseases may potentially explain the high cardiovascular outcomes and mortality among these patients (105).

Asthma patients have clearly more symptoms associated with OSA, such as snoring and shortness of breath, compared to OSA patients without asthma (109). In addition, the risk of having OSA is over 2-fold increased among asthma patients than asthma-free patients (101). It has also been found that treatment of OSA improves asthma symptoms significantly even among patients with asthma imbalance, and therefore unrecognized OSA may be a reason for persistent asthma symptoms (56, 110).

There is evidence that patients with IRD may be at increased risk for sleep disorders, particularly OSA (102). OSA might manifest as a comorbidity of these diseases by IRD affecting the temporomandibular joint. If the joint is affected, it may cause backward rotation of the lower jaw leading to narrowing of the upper airway (103).

2.1.1.6 Mortality

Severe OSA has been identified as an independent risk factor for mortality (51, 52). According to a meta-analysis of 17 studies concerning all-cause mortality with 681,072 deaths documented, severe OSA was associated with a 2.13-fold increased risk for all-cause mortality and, similarly, a subset of the data revealed a 2.73-fold risk for cardiovascular mortality (111). Importantly, long-term CPAP use showed reduced mortality rates among OSA patients (112).

2.1.1.7 Treatment and outcomes

Treatment of OSA requires long-term and multidisciplinary treatment. The treatment methods include conservative, medical, and surgical options.

2.1.1.7.1 Conservative treatment

Conservative treatment options are the most important part of care for OSA patients. These treatments include weight reduction, as it has been seen to reduce AHI in obese individuals. Weight loss may also benefit management of other existing comorbidities such as hypertension and high cholesterol (113). Exercising has been seen to lower AHI in addition to helping with weight control even without weight reduction (114). Positional therapy, avoiding a supine position during sleep,

can also reduce AHI significantly among patients who are suffering supine-position-related OSA (115). OSA patients should be advised on the importance of regular sleeping habits as sleep deprivation worsens OSA (116). These factors should be taken into consideration also when a patient has irregular working shifts (33).

Medications and substances that relax muscles or suppress respiratory drive (e.g., alcohol, benzodiazepines, and opioids) may also exacerbate OSA and should be minimized or avoided (79).

2.1.1.7.2 Continuous positive airway pressure

CPAP is the gold standard for OSA treatment in moderate or severe cases (117). CPAP directs a generally constant pressure to the upper airway, hence preventing pharyngeal collapse. CPAP has been shown to significantly decrease AHI (118). Patients with severe OSA benefit most from CPAP treatment. CPAP therapy significantly reduces both systolic and diastolic blood pressure, but with low effect size; 2.6±0.6 mmHg and 2.0±0.4 mmHg, respectively (83). Among other positive effects of CPAP treatment, it may also improve sleep-related symptoms and quality of life (119).

CPAP therapy's effects on cardiovascular outcomes and mortality rates range from significant prevention to non-significant findings (111, 120). However, a recent prospective cohort study showed that patients who had used a CPAP device for over 5 years were more than 5 times more likely to be alive at the end of the follow up (mean follow up 14 years) than non-CPAP users. In addition, long-term CPAP users were 1.74-times more likely to be alive than patients who had used a CPAP ≤ 5 years (112).

Despite several advantages of CPAP use, it may be problematic for some patients (121). Difficulty with a CPAP device may arise from a combination of mask discomfort, claustrophobia, pressure intolerance or lifestyle and social factors (118).

2.1.1.7.3 Mandibular advancement device

A mandibular advancement device (MAD) increases the upper airway volume during sleep by enlarging the pharyngeal airway and by preventing upper airway collapsibility. MAD covers the upper and lower teeth (the upper jaw may be toothless) and holds the mandible in a forward position during sleep (122). Mandibular protrusion advances the tongue position and subsequently increases oropharyngeal volume (123).

First line treatment for moderate and severe OSA is CPAP, but MADs may be used in patients who cannot tolerate CPAP and have a BMI under 30 kg/m^2 (30). In addition, MAD can be prescribed without a CPAP trial if a patient refuses CPAP therapy and AHI is under 30 events per hour and BMI under 30 kg/m^2 (33).

In patients with mild to moderate OSA, a reduction of AHI of over 50% was observed among 42.8% of MAD users (124, 125). In addition to the positive effects

to AHI, MAD treatment also reduces daytime sleepiness and improves quality of life (126). BMI has a significant impact on the success of MAD treatment and treatment outcomes are not as good among overweight individuals as in patients with normal weight (127). Leaner patients or patients with supine-dependent OSA have been reported to experience particular success with the device (126). There is evidence that individuals having asthma as a comorbidity of OSA would benefit from MAD treatment, as it has been shown to reduce asthma symptoms even in patients with imbalanced asthma (110).

2.1.1.7.4 Otolaryngologic surgeries

Among children and adolescents, hypertrophy of tonsillar and adenoid tissues can cause obstruction of the nasopharyngeal and oropharyngeal areas. Therefore, adenotonsillectomy or tonsillectomy can be considered first-line treatments for paediatric OSA (128, 129). In addition, it has been found that tonsil surgery has a positive effect on the growth direction of the mandible (67). Among adults, tonsillectomy may be a successful treatment for OSA, especially for patients with large tonsils and mild or moderate OSA (130).

Nasal obstruction can increase snoring and OSA. Intranasal pathology may increase resistance in the upper airway, which might lead to a subsequent collapse and cause hypopnoeas. Surgical techniques include septoplasty and removal of the concha bullosa, which may decrease airway resistance and can cure OSA in some and facilitate CPAP comfort for others (131).

The goal of using radiofrequency ablation (RFA) for treatment of OSA is tissue volumetric reduction (132). In one study, short term follow-up (under one year) showed a significant 31% reduction in the respiratory disturbance index (RDI) and, similarly, in ESS of 31%. After long-term follow-up (over 2 years) RDI showed a 45% reduction and ESS a 32% reduction (133). Tongue base RFA can be done alone or simultaneously with other upper airway surgical procedures. Clinically, the most common adjuvant procedure addresses palatal obstruction: uvulopalatopharyngoplasty (UPPP) (132).

The procedure of UPPP is designed to enlarge and stabilize the retro-palatal airway (134), but its efficacy in improving AHI is low, with around 41% reported success rate, although many patients improved subjectively (135). This seems to support the hypothesis that multiple levels of obstruction exist and explains why UPPP alone frequently results in a lack of symptom improvement.

Tongue-based surgeries are an option to treat lower pharyngeal obstruction with genioglossus advancement including hyoid suspension (136). The procedure is often an adjunctive intervention with UPPP, as success rates have been found to be higher in multiple-level surgeries than in tongue-based surgeries alone (76%, 70%, respectively) (137, 138).

As the obstruction of the upper airway may occur in multiple sites, patients may benefit from multilevel surgeries when treating OSA. These may include nasal, palatal, and tongue surgeries, and can be performed simultaneously or in a phased sequence (139, 140).

2.1.1.7.5 Orthognathic surgery

Maxillo-mandibular advancement is a skeletal surgery which relocates maxilla and mandible anteriorly by LeFort I and bilateral sagittal split osteotomy. In addition to bony structures, the procedure advances anterior pharyngeal tissues such as the base of tongue, the soft palate and suprahyoid musculature, creating more volume in the velo-oro-hypopharyngeal area (141). Suggested indications for this procedure include hypopharyngeal narrowing, velo-oro-hypopharyngeal narrowing, and possible retrognathia of the mandible or both maxilla and mandible, but the procedure is also adequate even in absence of craniofacial skeletal deformities (142).

The recommended minimum advancement of the mandible is 10 mm (143). The treatment is highly effective as, according to a meta-analysis, it reduces AHI by 76%, with a mean preoperative AHI of 53.4 and postoperative AHI of only 12.9. Additionally, for every 1 mm of mandibular advancement, the post-operative AHI was lowered by an average of 1.45 events/hour, and each 1mm advancement also induced a 0.5mm gain in pharyngeal airway space, which is defined as the minimum distance between the base of the tongue and the posterior pharyngeal wall (144). However, mandibular setback, which can be used to correct mandibular prognathism causing a relative narrowing of the upper airway, has not been seen to trigger OSA (145).

Jaw advancements may cause prognathism for patients with a normal preoperative maxillomandibular position and therefore aesthetic planning should be included when considering this surgical procedure. However, postoperative appearance is regarded as unsatisfactory by only 5.2% of patients who have undergone maxillomandibular advancement surgery (146, 147).

2.1.1.7.6 Bariatric surgery

Bariatric surgery is an effective treatment approach for obesity, with resultant improvement in obesity-related comorbidities such as OSA. OSA has been found to be improved or cured in 78% patients who undergo bariatric surgery, but moderate or severe OSA persist in 20% of patients after the operation. However, an important finding regarding OSA treatment is that AHI was found to be reduced significantly from 27.8 events per hour to 9.9 events per hour on average (148).

2.1.1.7.7 Hypoglossal nerve stimulation

A hypoglossal nerve stimulator protrudes the tongue by hypoglossal nerve stimulation, opening the pharyngeal airway. The device is implanted in the chest,

and it is connected to a wire that attaches to a small cuff on the hypoglossal nerve (149). The treatment can be considered for individuals over age 18 with AHI 15-65 events per hour, BMI under 32 and for patients who have been unable to tolerate CPAP or other treatment options (150). This method reduced AHI efficiently, showing a 68% improvement in a 3-year follow-up (151). However, it has also been reported that this method did not reduce AHI or ≥4% oxygen desaturation index (ODI) values significantly and weight gain was observed in patients during the treatment. In addition, the costs of the treatment may be high (152).

2.1.1.8 COVID-19 associations with obstructive sleep apnoea

OSA leads to a significant cost to individuals and society, especially because it is associated with higher risks of cardiometabolic diseases as well as increased mortality rates. During the year 2020 another serious respiratory-related disease - COVID-19 - emerged, increasing disease burden on both at the individual and health care levels. On this basis, concerns have been raised about the health burden of OSA and COVID-19 together, as both have effects on respiration, particularly through decreased oxygen saturation levels.

Coronaviruses are a large group of viruses that can infect both humans and animals. They cause mostly mild respiratory infections, but also serious life-threatening diseases such as severe acute respiratory syndrome and Middle East respiratory syndrome, and currently COVID-19. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a new virus in humans causing the respiratory illness COVID-19, with variation in the severity of the symptoms, spreading from person-to-person primarily through respiratory droplets and aerosol particles (14).

The first reported COVID-19 cases occurred in Wuhan, Hubei province, China, in late December 2019. The virus spread rapidly, and on March 11th, 2020 WHO made the assessment that COVID-19 was characterized as a pandemic. Most people with the virus recover well and spontaneously, but data from several countries have shown that 14% to 19% of infected individuals require hospital treatment (14). The reported hospitalisation rates have been highly age-dependent. Of all hospital admissions due to COVID-19, 0.4% have been aged 0–4 years, another 0.4% aged 5–17 years, 24.7% aged 18–49 years, 31.1% aged 50–64 years, and 43.4% aged ≥65 (153). In addition, the risk for in-hospital deaths were highly increased among the older age groups, showing an elevation of risk to 3.11-fold for 50-64 year olds, 5.77-fold for 65-74 year olds, 7.67-fold for 75-84 year olds and 10.98-fold for over 85 year olds compared to individuals aged 18-39 years (after adjusting for confounding factors, such as sex, obesity, diabetes and immunosuppression) (154).

Known risk factors for severe COVID-19 disease include male sex, older age, obesity, diabetes, cardiovascular disease, and decreased lung function (15). Therefore, severe COVID-19 and OSA share several common risk factors and

comorbidities. In addition, previous studies have suggested OSA is a risk factor for the severe form of COVID-19 (17-21). This risk might have a considerable effect on healthcare systems as OSA is a very common disease and a large fraction of the cases are undiagnosed. One study even suggested that OSA might increase the risk of contracting COVID-19 (18).

Several potential mechanisms for how OSA associates with COVID-19 have been suggested. First, OSA causes continuous low-grade inflammation (5), which may be of particular importance in obese patients as it may potentially contribute to the intensification of the cytokine storm that occurs in COVID-19 pneumonia (155). Second, OSA may predispose individuals to pneumonia due to upper airway microaspiration, which has been suggested as the main mechanism leading to viral pneumonia (156). Third, OSA may worsen the core symptoms of severe COVID-19, especially during the night, when decreased oxygen saturation levels occur in OSA (5).

2.2 Genetics of common complex diseases

A complex disease is defined as a trait affected by multiple genes and environmental factors. There are many diseases which fulfil this criteria, such as Alzheimer's disease, Parkinson's disease, kidney diseases and autoimmune diseases (157).

OSA is also a common complex disease; it is a mixture of genetics, lifestyle, and environmental factors which include socioeconomic status and diet. Genetically several genes predispose one to OSA (known as a polygenic disease), in contrast to monogenic diseases, which are caused by a single gene or a single variant. Polygenic diseases are caused by the common contribution of several independently-acting or interacting polymorphic genes. However, the contribution of each gene may be small or even unnoticeable. The presence of certain combinations of alleles can influence the occurrence of the disease and therapeutic efficacy of certain pharmaceutical drugs (158).

2.2.1 Human genetic variation

The human genome includes 3.2 billion nucleotides which are divided into chromosomes. There are 22 autosomal chromosomes and two sex chromosomes (the X chromosome and the Y chromosome). Normally, humans have two copies of each autosome and individuals with one copy of X and one of Y are male whereas individuals who have two copies of X are female.

Genetic information is carried in a sequence of nucleotides of deoxyribonucleic acid (DNA). Each molecule of DNA is a double helix formed from two

complementary strands of nucleotides held together by hydrogen bonds between cytosine (C) – guanine (G) and adenine (A) – thymine (T), Figure 4.

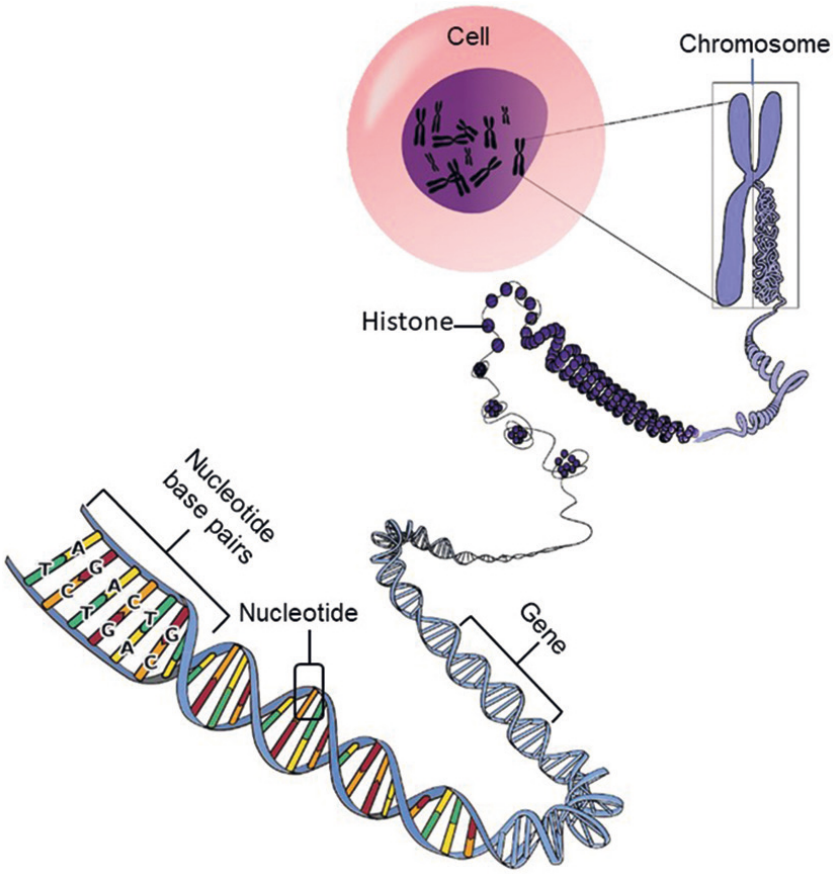

Figure 4. Structure of deoxyribonucleic acid (DNA). Chromosomes have proteins called histones that bind to DNA. DNA has two strands that twist into a helix. DNA is made up of four building blocks called nucleotides: adenine (A), thymine (T), guanine (G), and cytosine (C). The nucleotides attach to each other (A with T, and G with C) to form chemical bonds called base pairs, which connect the two DNA strands. The figure is adapted from pixabay.com under the Pixabay License.

Single nucleotide polymorphisms (SNPs) are points in the genome sequence where a sufficiently large fraction of the human population (e.g., >1%) has one nucleotide (e.g. an allele) while another large fraction has another. Therefore, an allele which is common in one geographical or ethnic group may be much rarer in another. At a location in the genome where there are two alleles, the SNP with lower frequency can be called the minor allele in a certain population, and its frequency is called the minor allele frequency.

Mapped sites in the human genome that are polymorphic, meaning that there is a reasonable probability that the genomes of two individuals will differ at this site, are extremely useful for genetic analyses. These analyses, including GWASes, compare altered allele frequencies between cases and controls or people with different phenotypes for a particular trait, for example for blood pressure.

A correlation structure exists throughout the human genome across genetic variation of different loci. This correlation structure allows us to use the genotype information at one locus to supply information about the genotype at another locus. This correlation between variation at different loci is called linkage disequilibrium (LD) (159). LD between two loci diminishes in proportion to the recombination rate and time when measured in generation numbers. Positive LD presents if two alleles are seen together on the same haplotype more often than expected, and negative LD presents if alleles are seen together on the same haplotype less often than expected (160). LD has implications in many methods of genetic research including population genetics and association studies (159).

2.2.1.1 Genetic characteristics of Finns

The Finnish population offers a unique environment for genetic research. This is mainly due to the small size of the indigenous population, the country's settlement history, the numerous so-called bottlenecks and centuries of geographical and cultural isolation with low amounts of immigration (161, 162). A bottleneck-effect occurs when a population's size is reduced, for example by a violent conflict. When the population expands after the event it is more genetically similar than before. Another special feature is due to settlement, showing that the genetic difference among Finns is relatively high between Eastern and Western parts of Finland. This genetic difference between Eastern and Western Finnish populations is even higher than that between German and British populations. Based on this unique history of the Finnish population and the enrichment of certain variants, a smaller number of Finns is sufficient to achieve adequate statistical power compared to a population where the disease would be very rare (163).

Importantly, studies have revealed a group of 36 monogenic diseases, called the Finnish disease heritage. These diseases are more frequent in Finland than in any other population, but on the other hand some diseases which are common in other populations do not exist among Finns (164, 165). These studies have not only highlighted the molecular mechanisms of Finnish diseases, but also showed information on the biological processes and metabolic pathways which are required for normal development and function of cells and tissues (165). In addition to the Finnish disease heritage, genetic variants enriched in the Finnish population have also been associated with many other diseases. For example, these variants have been linked with cholesterol levels and differences in levels of amino acids

in the blood, which are linked to several health problems including liver or kidney dysfunction in addition to changes in height and body weight (163, 166).

2.2.2 Genetic studies concerning obstructive sleep apnoea

Genetic studies provide a tool to identify independent genetic risk factors that modulate disease risk, and to examine causal pathways between comorbid traits.

Although the genetics of OSA is largely unstudied, reports have revealed that family members have a 2–4-fold greater risk of having OSA if there are OSA patients in the family. Although this can be caused partly by non-genetic factors, it is estimated that 40% of the variation in AHI is genetically regulated and can thus be explained by familial factors giving evidence of genetic predisposition to OSA (10). Twin and family studies have suggested that obesity, ventilatory responsiveness to hypoxemia and hypercapnia and craniofacial structures are strongly under genetic control as 30-70% of phenotypic variance has been explained by heritable factors (167-169). Presumably, genetic factors that contribute to the risk factors for OSA, such as craniofacial structures, body fat distribution and neural control, also have an effect on OSA predisposition (10). In addition, a study concerning over 4000 Finnish twins found that the concordance for snoring, the most common symptom of OSA, was greater between monozygotic twins than between dizygotic twins, further suggesting a role for inheritance (170).

2.2.2.1 Candidate gene association studies

Most genetic studies of OSA so far have been candidate gene association studies. The analyses in these studies depend on the hypothesis about the role of a selected gene on a certain phenotype. This single-gene method offers the advantage that predetermined genes can be prioritised and investigated first (171). Unfortunately, this method has given disappointing results, because there have been challenges replicating the findings and several studies have been underpowered (172). The limitations of the candidate gene approach are that inappropriate genes have been chosen for study and selection of genes relies on previous knowledge about disease mechanisms, which may prevent the finding of novel genetic variants in previously unknown pathways. Also, causative genes may be located either in the upstream or downstream signalling pathways of the selected genes (171).

In the case of OSA, one difficulty with the candidate gene strategy lies in selecting genes associated directly to OSA and not establishing genetic links related to comorbidities. However, studies have validated a few genetic associations with OSA through candidate gene methods. Certain associated and replicated SNPs have been found, including a meta-analysis proving an association between OSA and rs1800629 in the Tumor necrosis factor alpha gene (173). A multiple-cohort candidate gene study including individuals from both European and African-

American ancestry revealed two replicable SNPs associated with OSA: rs1409986 in the Prostaglandin E receptor 3 gene and rs7030789 in the Lysophosphatidic acid receptor 1 gene (174). In addition, the Apolipoprotein E ε4 locus has been investigated by several studies, but a meta-analysis concluded that the evidence is not strong enough to suggest causal association between the Apolipoprotein E ε4 locus and OSA (175). It has been observed that several previously identified genetic associations with OSA may be false positives (176).

2.2.2.2 Genome-wide linkage studies

The aim of genome-wide linkage studies is to use genotyped familial data to identify regions of the genome which are linked with the disease or a disease-associated phenotype without actually knowing the mutation in advance (177, 178). One genome-wide linkage analysis and follow-up association identified a connection between a marker of OSA severity and a polymorphism in the Angiopoietin-2 gene (179).

2.2.2.3 A short introduction to genome-wide association studies

A GWAS is an approach to study associations between genetic variants and diseases and/or traits in samples from populations. The primary goal is a better understanding of the biology of diseases, under the assumption that this will lead to better treatments or prevention. GWAS is facilitated by finding genetic regions with markers showing imbalance in their allele frequencies between disease cases and controls. These genetic regions are then further studied, or *finemapped*, to localize the likely candidate genes modifying the disease risk.

Knowledge of common genetic variation of the human genome is provided by two major research initiatives: The International HapMap Project and The 1000 Genomes project. The International HapMap Project has described the patterns of common SNPs within human DNA sequences, documenting recombination hotspots, a block-like structure of linkage disequilibrium and low haplotype diversity that leads to substantial correlations of SNPs with many of their neighbours (180). The 1000 Genomes project has created the largest public catalogue of human genetic variation and genotype data with the goal of finding most genetic variants with frequencies of at least 1% in the populations studied (181).

Haplotype block structure suggests that the recombination of chromosomes occurs only at a relatively small number of sites on the genome. These sites are called recombination hotspots (180). Phasing, which is a process of analysing known genotypes to infer haplotypes, can be performed accurately by collecting large numbers of genotyped samples and utilizing a probability model for estimating which are the most probable haplotypes in the study population. The accuracy is further improved by high quality reference data. Tag-SNPs are

representative SNPs in a region of the genome with high LD that represent a haploblock. Therefore, it is enough to genotype any tag-SNP of a causal SNP within a haploblock (182).

GWASes have been based on data from SNP arrays designed to tag common variants in the genome combined with stochastic imputation of non-genotyped markers using a reference panel from a set of whole-genome sequenced individuals. Modern genotyping arrays can genotype up to 10^6 markers simultaneously, while imputation may further increase the number of SNPs up to several millions. Imputation is a process of predicting genotypes that are not directly genotyped in a sample of individuals by using known haplotypes in a population, for instance from the HapMap or the 1000 Genomes Project (182). Importantly, it is one of the main uses of haplotype structure in GWAS.

Genome-wide association tests are used to identify regions of the genome associated with the phenotype of interest. For each SNP it is tested if the allele frequency significantly varies between the case and the control groups (183). Identification of trait-associated SNPs may subsequently reveal new insights into the biological mechanisms underlying these phenotypes. To date, genome-wide association studies have identified > 4,000 associations with diseases and traits (184).

2.2.2.3.1 Examples of genome-wide association studies

GWAS is nowadays a popular method when studying the genetics of common complex diseases. Below are represented brief examples of recent studies closely related to OSA comorbidities.

A meta-analysis has combined GWASes of T2D including 62,892 T2D cases and 596,424 controls of European ancestry with 16 million genetic variants. The study revealed 139 common and four rare variants, with 39 novel loci associated to T2D. These findings provided insights into the T2D aetiology, estimating the genetic structure of the trait and suggesting T2D is a polygenic disease. Both rare and common variants contribute to the genetic variation and it is indicated that rarer variants have larger effects on T2D risk (185).

It is established that obesity is heritable, and that it also predisposes one to many other diseases. To clarify the genetics of obesity utilizing BMI values, a GWAS was performed including 339,224 individuals. The study revealed 97 BMI-associated loci with 56 novel findings. Five of the loci showed evidence of several independent association signals, and many loci have significant effects on other metabolic phenotypes. The 97 loci explained 2.7% of BMI variation, and genome-wide estimates suggest that common variation accounts for over 20% of BMI variation. In addition, the role of the central nervous system in obesity susceptibility was suggested, implicating new genes and pathways including those

related to synaptic function, glutamate signalling, insulin secretion/action, energy metabolism, lipid biology and adipogenesis (9).

2.2.2.3.2 Genome-wide association studies concerning sleep apnoea

GWASes regarding OSA have previously identified associations with OSA severity, measured with AHI or respiratory event duration. These findings show an association to inflammatory, hypoxia signalling, and sleep pathways among Hispanic/Latino Americans, an association with AHI during non–rapid eye movement sleep in men, but not in women and identified genetic loci associated with variation in AHI over time (11-13). These findings are outlined in Table 3. Although these studies have provided the beginning of the OSA GWAS era, the results have not been replicable, with the exception of the finding at rs12936587, which was replicated in a small, independent physiological research study including 67 individuals (P=0.047) (13).

Table 3. Main findings of the previous genome-wide association studies (GWASes) for obstructive sleep apnoea-related traits

1st author	Trait	Sample size	GWAS finding	P
Tempaku, 2019	AHI, change over time	706	rs12415421 rs4731117	3.4×10^{-8} 4.4×10^{-8}
Chen, 2018	NREM AHI in men	Total 19744 Men 6737	rs12936587	1.7×10^{-8}
Cade, 2016	AHI, average respiratory event duration	12558	rs116791765 rs35424364	1.9×10^{-8} 4.9×10^{-8}

The main results of previous GWASes for OSA-related traits (11-13). AHI=apnoea-hypopnoea index, NREM=non-rapid eye movement sleep. The table is adapted from the manuscript Strausz et al. (2020) *Genetic analysis of obstructive sleep apnoea discovers a strong association with cardiometabolic health.*

3 AIMS OF THE STUDIES

This thesis concentrates on studying the role of OSA among Finns in cardiometabolic comorbidities, how genetic variation influences the risk for OSA, and whether there is an increased risk for severe disease when contracting novel respiratory viruses among OSA patients. The studies use epidemiological and longitudinal ascertainment, and also utilize modern genetic methods. The aims of the studies are the following:

I. To evaluate in a longitudinal prospective setting a) whether OSA modifies the risk of CHD and T2D independent of known risk factors such as BMI, blood pressure and lipids, b) the role of OSA in the development of diabetic complications including diabetic kidney disease (DKD) and c) whether OSA has similar effects in women and men.

II. To identify novel genetic loci associated with OSA risk and to test if OSA and its comorbidities, such as hypertension, T2D, CHD, stroke, depression, hypertension, asthma, and IRD, share a common genetic background.

III. To study whether OSA associates with the risk for severe COVID-19 disease independent of other potential risk factors including age, sex, BMI, hypertension, diabetes (including type 1 diabetes and T2D), CHD, asthma, and COPD, and also whether the risk for contracting COVID-19 is elevated among OSA patients.

4 MATERIALS AND METHODS

4.1 Healthcare system in Finland

The Finnish healthcare system is based on public healthcare services to which everyone residing in the country is entitled. According to the Constitution of Finland, the public authorities shall guarantee for every person adequate social, health and medical services (186).

Municipalities are responsible for organising and financing health care. Health services are divided into primary health care and secondary medical care. Primary health care services are provided at municipal health centres. Municipalities form hospital districts which are responsible for providing secondary health care in hospitals in their area. In addition, joint municipal authorities belong to five catchment areas for highly secondary health care, which are formed around the University Hospitals of Helsinki, Turku, Tampere, Oulu and Kuopio and at which the most demanding treatment is provided. Private health services complement municipal services and private operators provide both primary health care and secondary health care services (186).

Data is systematically collected from all these health care providers into nationwide registries maintained by governmental organizations such as the Social Insurance Institution of Finland, Statistics Finland and The Finnish Institute for Health and Welfare (THL) (187). Therefore, the Finnish healthcare system enables long-term monitoring of inhabitants independent of individual socioeconomic background and allows for follow-up of individuals for up to their whole lifespan, or for example during their growth period (188). This results in the ability to share information among healthcare professionals and higher quality of care, but is also highly useful for research purposes.

4.2 Study population

In all studies in this thesis a diagnosis of OSA is based on the International Classification of Diseases (ICD)-codes (ICD-10: G47.3, ICD-9: 3472A), which were obtained from the Finnish National Hospital Discharge Registry and the Causes of Death Registry. This diagnosis is based on subjective symptoms, clinical examination and sleep studies, applying the criteria of AHI/REI ≥ 5 per hour with associated signs/symptoms or medical/psychiatric disorder, or AHI/REI ≥ 15 per hour even in the absence of associated symptoms or disorders.

By combining ICD-codes from different registries, we generated the disease endpoints outlined in Table 4, which describes how endpoints were constructed for each phenotype.

Table 4. International Classification of Diseases (ICD)-codes for obstructive sleep apnoea (OSA) and comorbidities

Phenotype endpoint	ICD-10	ICD-9	ICD-8
OSA	G47.3	3472A	
Hypertension*	I10-I13, I15, I67.4	4019X, 4029A, 4029B, 4039A, 4040A, 4059A, 4059B, 4372A, 4059X	400, 401, 402, 403, 404
T2D**	E11	250A	
CHD	I20.0, I21, I22	410, 4110	410, 411,0
DKD	N18, N19, E10.2, E11.2	585, 2503A, 2503B	582,00, 250,04
Stroke	I61, I63, I64	431, 4330A, 4331A, 4339A, 4340A, 4341A, 4349A, 436	431, 433, 434, 436
Depression	F32, F33	2961, 2968	790,20, 298,0
Hypothyroidism	E00, E01, E02, E03.0-E03.5, E03.8, E03.9	243, 2443, 2448, 2449, 2448A, 2448B	243, 244
Asthma	J45, J46	493	493
IRD	M05, J99.0, M06.0, M30-M35, M45, M08.0, L40.5	7140A, 7140B, 7141, 7100, 7431, 7101, 7340, 7200, 7143A, 6960A	712,10, 712,4, 712,0, 696,00
Snoring	R06.5		

Phenotype endpoints were generated by combining ICD-codes from different registries. The Finnish national version for each ICD-code was used. These ICD-code criteria are all regular expressions for a hierarchical search. Hypertension* also includes Social Insurance Institution of Finland reimbursement code 205. T2D** includes also medication purchases for Anatomical Therapeutic Chemical code A10B, Blood glucose lowering drugs, excluding insulins. At least three separate purchases were required to ensure the correct diagnosis if diabetic medication was the only evidence. T2D=type 2 diabetes, CHD=coronary heart disease, DKD=diabetic kidney disease, IRD= inflammatory rheumatic diseases. The table is adapted from the manuscript Strausz et al. (2020) *Genetic analysis of obstructive sleep apnoea discovers a strong association with cardiometabolic health.*

4.2.1 FINRISK, Heath 2000 Survey, Botnia (Study I)

The population-based FINRISK surveys are independent random samples drawn from the population registry of six geographic areas of Finland (North Karelia, Kuopio, Lapland, Oulu, Turku/Loimaa and Helsinki/Vantaa) and stratified

according to gender, 10-year age group and study area. The study included a questionnaire in addition to a clinical examination at which a blood sample was drawn (189). Participants from different survey years (1992, 1997, 2002 or 2007) were pooled together.

The total sample size for all FINRISK surveys was 29,257 and participants who had missing information (N=7) or type 1 diabetes (T1D) (N=297) were excluded from the study. Thus, the total sample size was 28,953 where 13,792 male and 15,161 female participants aged 24–74 years at baseline were included in the analyses. Of these participants, 1214 (4.2%) had OSA.

The Health 2000 Survey (H2000) is a comprehensive combination of a health interview and a health examination survey. The study was based on a nationally representative sample of 8,028 persons aged ≥30 years living in mainland Finland (190). After excluding participants who had missing information (N=1,331) or T1D (N=92), the final dataset consisted of 6605 participants, 2940 men and 3707 women. Out of this cohort 235 (3.6%) participants were diagnosed with OSA.

The Botnia Study was established in 1990 to investigate familial clustering of diabetes in the Ostrobothnia region in western Finland, and the non-diabetic participants have been prospectively followed (191). The population-based PPP-Botnia Study was conducted in the same geographical area (192). From the Botnia/PPP Botnia Studies (referred to collectively as the Botnia study), we included 1,405 patients with T2D, 735 men and 670 women. In this cohort, 119 participants (8.5%) had an OSA diagnosis.

During the follow-up of the study cohorts, data for hospitalisations and causes of death were obtained from the Finnish National Hospital Discharge Registry and the Causes of Death Registry. Follow-up for FINRISK ended on 31 December 2014, for H2000 on 31 December 2013 and for Botnia on 31 December 2015.

In the FINRISK cohorts, the follow-up was up to 22 years (median 12.9 years, interquartile range ((IQR) 8.5–17.9) and in the H2000 cohort the follow-up was up to 14.5 years (median 13.9, IQR 13.6–14.2). In the the Botnia study, the follow-up was up to 25 years (median 14.7 years, IQR 10.2–21.4). Altogether, we had 523,372 person-years of follow-up.

4.2.2 FinnGen (Study II-III)

The FinnGen study (www.finngen.fi/en) is a remarkable biobank study that aims to genotype 500,000 Finns. The data includes prospective and retrospective epidemiological and disease-based cohorts as well as hospital biobank samples. The FinnGen study combines genomic data with longitudinal registry data that records health care events over the entire lifespan of an participant. This includes data from the National Hospital Discharge Registry (available from 1968), Causes of Death Registry (available from 1969), the National Infectious Diseases Registry

(available from 1995), Cancer Registry (available from 1953) and Medication Reimbursement Registry (available from 1995), all utilizing unique national personal identification codes. Registry data was available from the beginning of the registry until 31.12.2018, (Figure 5). In study II, the data from FinnGen Data Freeze 5 consisted of 218,792 individuals. Participants who had ICD-code G47 (Sleep disorders) were excluded from the controls (N=837) and thus the remaining sample size was 217,955 participants. Of these, 94,799 were men and 123,156 were women, including 16,716 (7.7%) which were OSA patients. In study III, the data consisted of 260,405 FinnGen Data Freeze 6 participants, 113,344 men and 147,061 women, including 20,279 OSA patients. From that data individuals with polymerase chain reaction (PCR)-validated COVID-19 diagnosis were derived (N=445).

4.2.2.1 Data security in FinnGen

The FinnGen study manages healthcare registry and genomic data without compromising the privacy and integrity of participants. A key feature of the project is that the pseudonymized individual-level data is released into a secure, carefully monitored environment with limited access rights that does not allow internet access nor download of the individual-level data to local environments. The members of the research project only handle data that has been pseudonymized and no strong identifiers are linked to the data (e.g. social security numbers, emails, addresses). In addition, very rare clinical endpoints (less than 5 occurrences) are removed, and the time of events is randomized (+- 15 days).

The University of Helsinki is responsible for the FinnGen research project and is the official data controller of the study. In addition, the FinnGen study has nominated persons responsible for information security and data protection, whose job is to supervise the information security and data protection of the research subjects.

The FinnGen information technology environment and data protection practices are regularly reviewed by impartial third-party service providers. The full data protection statement can be found here: https://www.finngen.fi/en/data_protection/data-protection-statement.

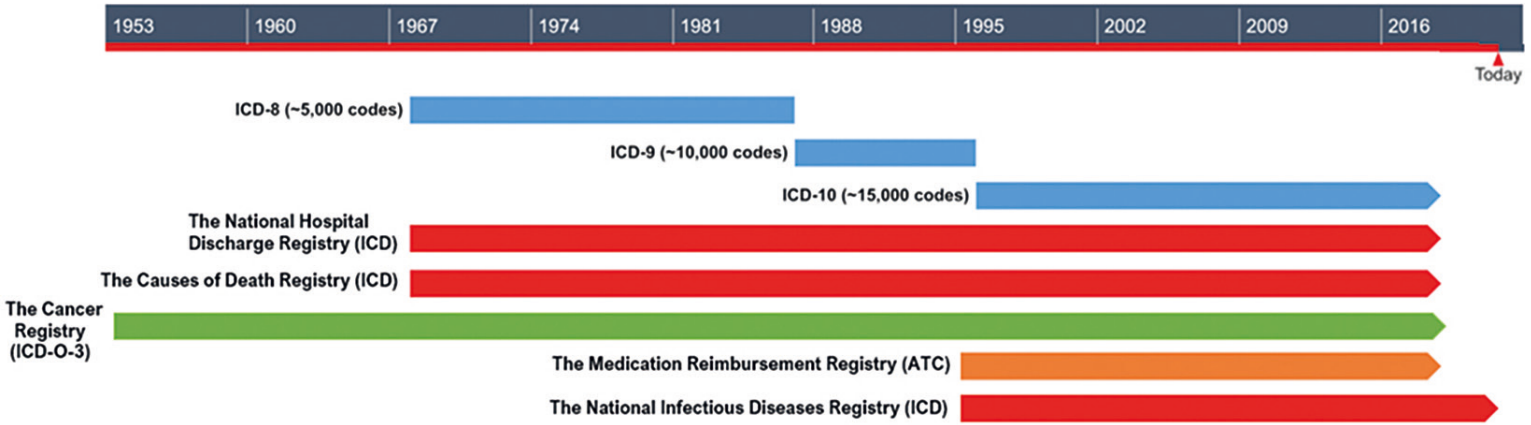

Figure 5. Nationwide registries combined by into the FinnGen study. X-axis represents the date a certain registry collection was started. Each arrow on the Y-axis shows the origin of the International Classification of Diseases (ICD)- or Anatomical Therapeutic Chemical Classification System (ATC)-code. ICD-O-3=International Classification of Diseases for Oncology, 3rd Edition. The figure is adapted from the manuscript Strausz et al. (2020) *Genetic analysis of obstructive sleep apnoea discovers a strong association with cardiometabolic health.*

4.2.3 International replication cohorts (Study II)

The UK Biobank (UKBB, https://www.ukbiobank.ac.uk/), Estonian Biobank (ESTBB) www.biobank.ee) and All New Diabetics in Scania (ANDIS, http://andis.ludc.med.lu.se/) cohorts were utilized to replicate our GWAS results and were used in forming a meta-analysis concerning the main results of Study II. Data with full phenotype and genotype information and individuals passing genotyping quality control were included in the analyses.

The UKBB is a large national and international health resource, with the aim of enhancing the prevention, diagnosis, and treatment of a wide range of serious and life-threatening illnesses. UKBB recruited 500,000 people in 2006-2010 from across the United Kingdom. OSA diagnosis was based on ICD-10: G47.3. The study sample in the UKBB included 4,471 OSA cases and 403,723 controls.

The ESTBB is a population-based biobank of the Estonian Genome Center at the University of Tartu. The cohort size is currently close to 150,000 participants. Patients were selected by ICD-10: G47.3. For additional confirmation of the diagnosis, treatment service codes from the Health Insurance Fund were also used. The study sample in the ESTBB included 4,930 OSA patients and 61,056 age- and sex-matched controls.

The ANDIS study aims to recruit all incident cases of diabetes within Scania County in Southern Sweden. All health care providers in the region were invited; the current registration covered 14,625 patients. OSA was defined by ICD-10: G47.3. The study sample included 947 OSA patients and 9,829 controls.

4.2.4 Hospital District of Helsinki and Uusimaa hospital's patients records (Study II and III)

For the validation of OSA diagnoses, 1,000 OSA patients were randomly selected among patients who were treated in Hospital District of Helsinki and Uusimaa (HUS) during the years 2008-2011 and 2016-2019. The diagnoses were derived from HUS's Hospital Discharge Registry and confirmed utilizing individual-level medical records.

Treatment information concerning the association between OSA and COVID-19 was collected from the patient records of the Heart and Lung Center or Department of Oral and Maxillofacial Diseases, Helsinki University Hospital (HUH), Finland.

4.3 Genotyping and imputation (Study II)

The FinnGen study genotyped samples with Illumina and Affymetrix arrays (Thermo Fisher Scientific, Santa Clara, California, USA). Genotype calls were made with the GenCall and zCall -algorithms for Illumina and the AxiomGT1 algorithm for Affymetrix chip data. Genotyping data produced with previous chip platforms were lifted over to human reference genome build version 38. Samples with sex discrepancies, missingness (>5%), excess heterozygosity (+-4 standard deviation (SD)) and non-Finnish ancestry were removed. Variants with high missingness (>2%), deviation from Hardy–Weinberg equilibrium ($P<1.0\times10^{-6}$) and low minor allele count (<3) were removed. Pre-phasing of genotyped data was performed with Eagle 2.3.5 (https://data.broadinstitute.org/alkesgroup/Eagle/) with default parameters, excluding the number of conditioning haplotypes which was set to 20,000. Imputation was done using the population-specific Sequencing Initiative Suomi (SISu) v3 imputation reference panel with Beagle 4.1 (version 08Jun17.d8b, https://faculty.washington.edu/browning/beagle/b4_1.html) as described in the following protocol: [dx.doi.org/10.17504/protocols.io.nmndc5e]. The SISu v3 imputation reference panel was developed using high-coverage (25–30×) whole-genome sequencing data generated at the Broad Institute of MIT and Harvard and at the McDonnell Genome Institute at Washington University, USA and was jointly processed at the Broad Institute. The variant callset was produced with genomic analysis toolkit (GATK) HaplotypeCaller algorithm by following GATK best-practices for variant calling. Genotype-, sample- and variant-wise quality control was applied in an iterative manner by using the Hail framework v0.1 (https://Github.com/hail-is/hail/releases/tag/0.2.13, http://Doi.org/10.5281/zenodo.2646680).

The resulting high-quality whole genome sequence data for 3775 individuals were phased with Eagle 2.3.5 as described above. Post-imputation quality control involved excluding variants with imputation quality score (INFO) <0.7.

4.4 Statistical analyses

To study OSA comprehensively and accurately, a wide range of statistical methods were utilized. These include basic analyses, but also the latest statistical genetics approaches in addition to longitudinal analyses.

4.4.1 General analyses

To test and evaluate the differences in baseline demographics and clinical characteristics, χ^2 tests were used, unless the expected cell size was ≤5 where

Fisher's exact test was used instead. For continuous variables, Student's t-test was utilized. $P<0.05$ was considered statistically significant, and all tests were two sided. Logistic regression analysis was used to calculate odds between the dependent binary variables and the sets of independent variables such as age, sex and OSA comorbidities.

4.4.2 Prospective analyses

The associations between OSA and incident CHD events, DKD events and T2D were tested using Cox proportional hazards models implemented in the R package Survival. Age at onset of OSA was used as a time-dependent covariate in the analyses and age was used as the timescale. In such a Cox model, a person contributes to the model only for his/her at-risk period (i.e., for a certain age range). During that period, the individual could become an OSA case, and before could have a T2D diagnosis or a cardiovascular event. In this case, using OSA as a time-dependent covariate, a participant contributes to the model as a non-OSA case until the age at OSA diagnosis, and as an OSA case for the remainder of his/her at-risk period. Prevalent cases were excluded from the Cox regression analyses and the assumptions of the models were tested with the cox.zph-function using scaled Schoenfeld residuals.

In our FINRISK unadjusted model for CHD, we used age, gender, geographical area and cohort year as covariates. In the adjusted model, in addition to aforementioned factors, traditional risk factors were used as covariates for cardiovascular events: HDL, total cholesterol, current cigarette smoking, BMI, hypertension (defined as a measured blood pressure of at least 140/90 mm Hg or the use of antihypertensive medications), prevalent T2D and family history of stroke or myocardial infarction.

In the unadjusted analysis, similar to CHD, the association between OSA and T2D was adjusted for age, gender, geographical area and cohort year. In the adjusted model, also BMI was used as a covariate. Among patients with T2D with the endpoint of DKD, the model was adjusted for BMI and hypertension.

In the H2000 cohort, it was not possible to adjust the model for family history of stroke or myocardial infarction because that information was not determined in the study. Otherwise, the Cox time-dependent hazards model was adjusted for the same risk factors as mentioned previously.

The evidence from the FINRISK and H2000 cohorts were combined to analyse CHD and T2D. To analyse T2D complications in more detail, the Botnia study was used as a third cohort. The results were combined using fixed-effect meta-analysis.

4.4.3 Genome-wide association testing

A GWAS is a method used in genetics research to link specific genetic variations with certain diseases. The approach requires examining the genomes from several individuals and looking for genetic markers which may be used to predict the presence of a disease. Finding such genetic markers can lead to a better understanding of how genes contribute to disease and the results may guide the development of prevention and treatment strategies (193).

A total of 218,792 samples from FinnGen Data Freeze 5 with 2,925 disease endpoints were analyzed using scalable and accurate implementation of generalized mixed model (SAIGE), which uses saddle point approximation to calibrate unbalanced case-control ratios. In addition, this method reduces the risk for type 1 error (194).

Analyses were adjusted for current age or the age at death, sex, genotyping chip, genetic relationship and first the 10 principal components (PCs). For OSA, a GWAS was performed in a similar manner (N=217,955, including 16,761 OSA patients and 201,194 controls), but was also adjusted for BMI (N=159,731, including 12,759 OSA patients and 146,972 controls).

For the replication of the FinnGen OSA GWAS results, evidence from the UKBB, ESTBB and ANDIS cohorts were merged. The results were combined using inverse-variance weighted fixed-effect meta-analysis using beta estimates and beta standard errors with the R package Metagen as implemented in R version V.4.0.2 (www.r-project.org). The merged data consisted of 10,348 OSA cases and 474,608 controls.

4.4.4 Phenome-wide association testing

Phenome-wide association studies (PheWASes) can identify pleiotropy, or the finding of multiple independent phenotypes associated with a single genetic variant. GWAS and PheWAS approaches are complementary, with the ability to replicate and validate the other's findings (195, 196). A PheWAS was performed using the FinnGen data which examined the associations between the lead SNPs in OSA GWASes and 2,925 disease endpoints. The PheWAS method was also utilized to study the association between lead variants and drug purchases.

4.4.5 Meta-analyses

To combine and generalize the results of our studies we used meta-analyses. Fixed-effect meta-analysis was utilized if the heterogeneity between studies was not statically significant ($P > 0.1$). Otherwise, random-effect meta-analysis was implemented with the restricted maximum likelihood method (REML) to estimate the heterogeneity of variance (197).

4.4.6 Polygenic risk score and Mendelian randomization

Polygenic risk scores (PRSes) summarize the effects of genome-wide genetic markers to measure the genetic liability of a trait or a disorder. Studies based on PRSes have revealed promising results when predicting complex traits and diseases. The results may be utilized in early diagnosis of disease, risk stratification, and also in disease prevention (198, 199).

PRSes for BMI were calculated using summary statistics for 996,250 variants (9). The posterior effect sizes were calculated utilizing the PRS-continuous shrinkage (CS) method. PRS-CS is a polygenic prediction approach which infers posterior effect sizes of SNPs using genome-wide association summary statistics in addition to an external LD reference panel (200).

PRSes for the OSA replication were calculated using dosage-weighted beta-estimates for each lead variant ($P<5\times10^{-8}$, 5 variants). The scores were generated using Plink2 (https://www.cog-genomics.org/plink/2.0/) in the FinnGen data and the weights from FinnGen were projected to the UKBB individual-level data.

Mendelian randomization (MR) is a method which uses genetic variation to improve causal assumptions in observational studies. A genetic variant linked with the exposure of interest (a genetic instrument) is utilized to test the causal relationship between exposure and outcome. If the association exists, a causal relationship can be assumed. It is assumed, in contrast to observational associations, that the genetic variant is not subject to issues of reverse causation and/or confounding (201). The assumptions of MR include the following: 1) the genetic instrument is associated with the exposure of interest, 2) the genetic instrument is independent of factors that confound the association of the exposure and the outcome and 3) the genetic instrument is independent of the outcome, given the exposure and the confounders (202).

MR analysis was performed to investigate the causality between BMI and OSA using independent BMI SNPs (9). A genetic variant associated with the exposure of interest was used to test the causal relationship with the exposure (BMI) and outcome (OSA).

4.4.7 Genetic correlations

Genetic correlation between traits is a measure of the genetic components shared by traits. To estimate genetic correlations, SNP-based heritability and tissue-specific SNP-heritability LD score regression (LDSC) was utilized. The approach quantifies the contribution of each variant by examining the relationship between test statistics and LD (203). SNP heritability is defined as the fraction of the phenotypic variance explained by the additive effects of a given set of genetic variants (204, 205). In calculating LD scores, a subset of the 1000 Genomes Project derived from European ancestry individuals was used (181). To restrict

to a set of common, well-imputed variants, only those SNPs in the HapMap 3 reference panel were retained (206).

Summary statistics from the FinnGen data were used to study genetic correlations between OSA, BMI, hypertension, T2D, CHD, stroke, depression, hypothyroidism, asthma and IRD. For sleep traits, summary statistics derived from the UKBB data were used, where study subjects self-reported snoring (207), sleep duration, sleepiness (208) and chronotype (209).

Sleep efficiency (sleep duration divided by the time between the start and end of the first and last nocturnal inactivity period, respectively) was based on accelerometer-derived measures (210).

For tissue-specific SNP-heritability a method (211, 212) combining data from Encyclopaedia of DNA Elements (ENCODE, https://www.encodeproject.org/) and the Genotype-Tissue Expression (GTEx, https://gtexportal.org/home/) resources was used.

4.4.8 Gene based analysis

Gene-based tests were performed using multi-marker analysis of genomic annotation (MAGMA) as implemented in the Functional mapping and annotation platform. Analyses provide aggregate associated P-values based on all variants located within a gene and its regulatory region using information from 18 biological data repositories and tools (213). This analysis included a gene-based test to detect significant SNPs associated with OSA using FinnGen OSA summary statistics.

4.5 Ethics statement

FINRISK data are stored in THL Biobank which distributes them to researchers on the basis of written applications. The Coordinating Ethical Committee of the Helsinki and Uusimaa Hospital District approved our THL Biobank request with the decision # 238/13/03/00/2014. The H2000 protocol was approved by the Ethical Committee of the National Public Health Institute (decision number 8/99). The Botnia Study protocols were approved by the Ethics Committee of the Helsinki University Central Hospital, Finland, with the decision #574/E5/03.

Patients and controls in FinnGen provided informed consent for biobank research, based on the Finnish Biobank Act. Alternatively, older research cohorts collected prior to the start of the FinnGen study (August 2017), were collected based on study-specific consents and later transferred to the Finnish biobanks after approval by Valvira, the National Supervisory Authority for Welfare and Health. Recruitment protocols followed the biobank protocols approved by Valvira. The

Coordinating Ethics Committee of HUS approved the FinnGen study protocol Nr HUS/990/2017.

5 RESULTS

5.1 Validation of obstructive sleep apnoea diagnosis

OSA diagnosis was validated using HUS's Hospital Discharge Registry by collecting information from 1,000 patients and comparing the registry data to the patient's medical records. OSA diagnosis had a validity of over 98% positive predictive value (PPV), (Figure 6).

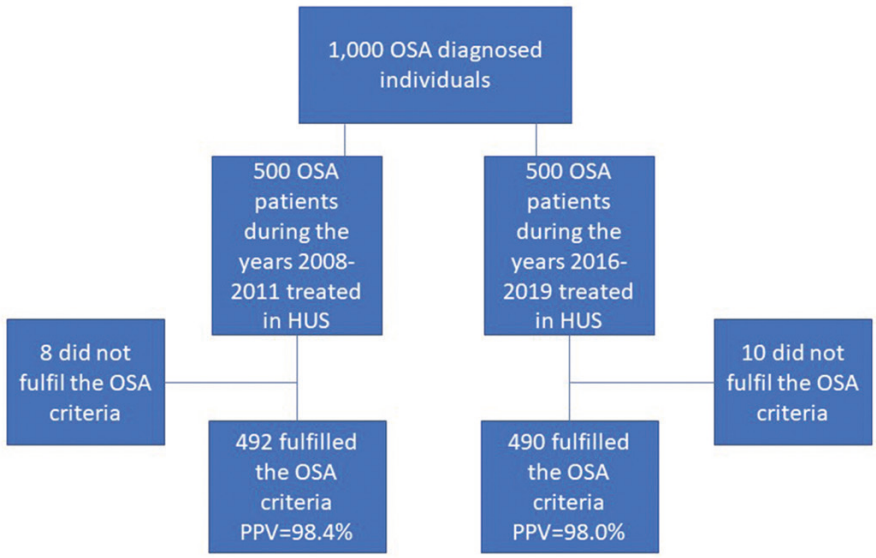

Figure 6. Obstructive sleep apnoea (OSA) diagnosis was validated using Hospital District of Helsinki and Uusimaa (HUS) Hospital Discharge Registry, collecting information from 1,000 patients and comparing the registry data to the patient medical records. OSA diagnosis has a validity of over 98% positive predictive value (PPV) when using the International Classification criteria for Sleep Disorders for OSA (44). The figure is adapted from the manuscript Strausz et al. (2020) *Genetic analysis of obstructive sleep apnoea discovers a strong association with cardiometabolic health.*

Diagnosis of OSA was confirmed using the International Classification criteria for Sleep Disorders, which requires either signs/symptoms (e.g. associated sleepiness, fatigue, insomnia, snoring, subjective nocturnal respiratory disturbance, or observed apnoea) or associated medical or psychiatric disorder (i.e. hypertension, coronary artery disease, atrial fibrillation, congestive heart failure, stroke, diabetes, cognitive dysfunction, or mood disorder) coupled with five or more predominantly obstructive respiratory events per hour. Alternatively, a frequency of obstructive respiratory events 15 per hour satisfies the criteria, even in the absence of associated symptoms or disorders (44).

5.2 Associations between obstructive sleep apnoea and incident cardiometabolic diseases

To analyse the comorbidity of OSA and CHD, T2D outcomes and T2D complications, longitudinal data from three population-based cohorts were combined including 36,963 participants with 1,568 (4.2%) patients with OSA. These cohorts included FINRISK (N=28,953) with a follow-up of up to 22 years (median 12.9 years, IQR 8.5–17.9), H2000 (N=6,605) with a median follow-up of 13.9 years (IQR 13.6–14.2) and patients with T2D from the Botnia Study (N=1,405) with a median follow-up of 15.3 years (IQR 10.8–21.3). Altogether the sample included 6,248 patients with T2D (16.9%). Baseline demographics and clinical characteristics are outlined in Table 5.

Table 5. Baseline characteristics in FINRISK, Health 2000 Survey (H2000) and patients with type 2 diabetes (T2D) in Botnia

	FINRISK				H2000				Botnia T2D			
	Overall	Non-OSA	OSA	P	Overall	Non-OSA	OSA	P	Overall	Non-OSA	OSA	P
	N_{all}=28953	N_{all}=27739	N_{all}= 1214		N_{all}=6605	N_{all}=6370	N_{all}=235		N_{all}=1405	N_{all}=1286	N_{all}=119	
	N_{T2D}=4006	N_{T2D}=3634	N_{T2D}=372		N_{T2D}=837	N_{T2D}=773	N_{T2D}=64		N_{T2D}=1405	N_{T2D}=1286	N_{T2D}=119	
Sex (male) (N, %)	13792 (47.6)	12915 (46.6)	877 (72.2)	1.26×10^{-68}	2940 (44.6)	2768 (43.5)	172 (73.2)	3.8×10^{-19}	735 (52.3)	651 (50.6)	84 (70.6)	4.6×10^{-5}
Baseline age (mean in years, SD)	48.01 (13.2)	47.95 (13.3)	49.27 (11.3)	8.2×10^{-5}	53.8 (15.7)	53.9 (15.8)	50.7 (10.5)	9.7×10^{-6}	58.94 (11.5)	59.20 (11.6)	56.1 (9.9)	1.6×10^{-3}
Age at OSA diagnosis (mean in years, SD)			55.30 (10.4)				55.81 (10.5)				61.93 (10.7)	
BMI (mean kg/m^2, SD)	26.74 (4.7)	26.58 (4.5)	30.34 (5.7)	3.5×10^{-96}	26.9 (4.7)	26.8 (4.59)	30.6 (5.74)	1.5×10^{-20}	29.26 (4.8)	28.99 (4.7)	32.20 (4.9)	6.1×10^{-10}
Current smoking (N, %)	6978 (24.2)	6666 (24.1)	312 (25.8)	0.2	1397 (21.3)	1340 (21.2)	57 (24.4)	0.27	193 (13.7)	165 (12.8)	28 (23.5)	1.9×10^{-3}
Systolic BP (mean mmHg, SD)	135.7 (20.0)	135.6 (20.1)	137.0 (17.5)	7.8×10^{-3}	135.0 (21.70)	135.0 (21.8)	136.1 (19.2)	0.41	144.6 (20.4)	144.6 (20.4)	145.0 (21.1)	0.83
Diastolic BP (mean mmHg, SD)	80.48 (11.6)	80.33 (11.6)	83.7 (11.2)	6.4×10^{-25}	81.7 (11.30)	81.5 (11.3)	86.5 (10.2)	2.8×10^{-12}	84.4 (10.4)	84.0 (10.3)	87.9 (10.3)	1.3×10^{-4}
CHOL (mean mmol/l, SD)	5.51 (1.1)	5.51 (1.1)	5.56 (1.0)	0.07	5.9 (1.1)	5.9 (1.1)	6.0 (1.1)	0.12	5.5 (1.1)	5.6 (1.1)	5.3 (1.0)	3.4×10^{-3}
LDL (mean mmol/l, SD)	3.352 (0.9)	3.34 (0.9)	3.50 (0.8)	1.0×10^{-3}	3.7 (1.1)	3.7 (1.1)	3.8 (1.0)	0.29	3.2 (1.0)	3.2 (1.0)	3.1 (0.9)	0.50
HDL (mean mmol/l, SD)	1.436 (0.4)	1.44 (0.4)	1.28 (0.3)	9.0×10^{-53}	1.3 (0.4)	1.3 (0.4)	1.2 (0.36)	9.7×10^{-5}	1.2 (0.3)	1.2 (0.3)	1.2 (0.3)	3.3×10^{-3}

	FINRISK				H2000				Botnia T2D			
Prevalent cases												
CHD (N, %)	749 (2.6)	691 (2.5)	58 (4.8)	1.4×10^{-6}	242 (3.7)	237 (3.7)	5 (2.1)	0.27	43 (3.1)	38 (3.0)	5 (4.2)	0.41
Stroke (N, %)	324 (1.1)	311 (1.1)	13 (1.1)	0.98	167 (2.5)	166 (2.6)	1 (0.4)	0.06	21 (1.5)	20 (1.6)	1 (0.8)	1
T2D (N, %)	1525 (5.3)	1403 (5.1)	122 (10.0)	4.2×10^{-14}	381 (5.8)	362 (5.7)	19 (8.1)	0.16	1018 (72.5)	938 (72.9)	80 (67.2)	0.06
DKD (N, %)	20 (0.1)	20 (0.1)	0	1	5 (0.1)	5 (0.1)	0	1	3 (0.2)	2 (0.2)	1 (0.8)	0.23
CHD/T2D (N, %)	238 (5.9)	214 (5.9)	24 (6.5)	0.74	63 (7.5)	62 (8.0)	1 (1.6)	0.08	43 (3.1)	38 (3.0)	5 (4.2)	0.41
DKD/T2D (N, %)	9 (0.2)	9 (0.2)	0	1	2 (0.2)	2 (0.3)	0	1	3 (0.2)	2 (0.2)	1 (0.8)	
Incident cases												
CHD (N, %)	2181 (7.5)	2035 (7.3)	146 (12.0)	1.9×10^{-9}	576 (8.7)	546 (8.6)	30 (13.3)	0.03	254 (18.2)	230 (18.0)	24 (20.1)	0.64
Stroke (N, %)	1325 (4.6)	1264 (4.6)	61 (5.0)	0.49	352 (5.3)	338 (5.3)	14 (6.0)	0.77	179 (12.8)	162 (12.7)	17 (14.3)	0.72
T2D (N, %)	2481 (8.8)	2231 (8.0)	250 (20.6)	2.0×10^{-52}	456 (6.9)	411 (6.5)	45 (19.1)	1.3×10^{-13}	387 (27.5)	348 (27.1)	39 (32.8)	0.10
DKD (N, %)	296 (1.0)	262 (0.9)	34 (2.8)	7.9×10^{-10}	112 (1.7)	109 (1.7)	3 (1.3)	0.80	91 (6.5)	77 (6.0)	14 (11.8)	0.03
CHD/T2D (N, %)	657 (16.4)	584 (16.1)	73 (19.6)	0.10	154 (18.4)	141 (18.2)	13 (20.3)	0.81	254 (18.2)	230 (18.0)	24 (20.1)	0.64
DKD/T2D (N, %)	151 (3.8)	128 (3.5)	23 (6.2)	0.02	43 (5.1)	42 (5.4)	1 (1.6)	0.24	91 (6.5)	77 (6)	14 (11.8)	0.03

P-values of baseline demographics and clinical characteristics were based on χ^2 tests. Fisher´s exact-test was used if the sample size was ≤ 5. For continuous variables a Student's t-test was used. OSA=obstructive sleep apnoea, BMI=body mass index, BP=blood pressure, CHOL=total cholesterol, LDL=low density lipoprotein, HDL=high density lipoprotein, CHD=coronary heart disease, T2D=type 2 diabetes, DKD=diabetic kidney disease. CHD/T2D=coronary heart disease among type 2 diabetic patients. DKD/T2D=diabetic kidney disease among type 2 diabetic patients, SD=standard deviation. The table is adapted from the manuscript Strausz et al. (2018) *Obstructive sleep apnoea and the risk for coronary heart disease and type 2 diabetes: a longitudinal population-based study in Finland*.

5.2.1 Cardiovascular outcomes

The association between OSA and the risk of incident CHD was evaluated. The unadjusted model was adjusted for age, sex and geographical region. OSA diagnosis was associated with an increased risk of CHD (hazard ratio (HR)=1.54, 95% confidence interval (CI) 1.28 to 1.86, $P=4.43\times10^{-6}$).

Table 6. Association between obstructive sleep apnoea and incident coronary heart disease events

	Number of events/ Subjects at risk	Unadjusted model		Adjusted model	
All		HR [95% CI]	P	HR [95% CI]	P
FINRISK	2129/27948	1.43 [1.17-1.75]	7.34×10^{-4}	1.25 [1.01-1.54]	0.037
H2000	565/6267	2.13 [1.40-3.24]	4.08×10^{-4}	1.91 [1.25-2.92]	2.80×10^{-3}
Combined	2694/34215	1.54 [1.28-1.86]	4.43×10^{-6}	1.36 [1.12-1.64]	1.40×10^{-3}
Men					
FINRISK	1480/13066	1.33 [1.06-1.67]	0.015	1.18 [0.94-1.49]	0.157
H2000	306/2748	1.81 [1.13-2.91]	0.014	1.57 [0.97-2.55]	0.069
Combined	1786/15814	1.41 [1.15-1.73]	1.10×10^{-3}	1.25 [1.01-1.54]	0.039
Women					
FINRISK	649/14882	1.99 [1.24-3.19]	4.11×10^{-4}	1.66 [1.03-2.68]	0.036
H2000	259/3519	4.12 [1.68-10.18]	2.06×10^{-3}	4.03 [1.62-10.01]	2.64×10^{-3}
Combined	908/18401	2.33 [1.53-3.53]	7.19×10^{-5}	2.01 [1.31-3.07]	1.20×10^{-3}

The FINRISK unadjusted model is adjusted for age, cohort year, geographical area and gender. The adjusted model is adjusted for high density lipoprotein (HDL) and total cholesterol (CHOL), current cigarette smoking, body mass index (BMI), hypertension, prevalent type 2 diabetes and family history of stroke or myocardial infarction in addition to covariates of the unadjusted model. The Health 2000 Survey (H2000) unadjusted model is adjusted for geographical area and gender. H2000-adjusted model is adjusted for HDL and CHOL, current cigarette smoking, BMI, hypertension, and prevalent type 2 diabetes in addition to covariates of the unadjusted model. HR=hazard ratio, CI=confidence interval. The table is adapted from the manuscript Strausz et al. (2018) *Obstructive sleep apnoea and the risk for coronary heart disease and type 2 diabetes: a longitudinal population-based study in Finland*.

In addition, the model was adjusted for CHD risk factors including age, sex, region, HDL and total cholesterol, current cigarette smoking, BMI, hypertension, T2D baseline and family history of stroke or myocardial infarction. As a result, this association was somewhat attenuated (adjusted HR=1.36, 95% CI 1.12 to 1.64, $P=1.40\times10^{-3}$).

The estimates were concordant across the cohorts and were slightly higher for women (adjusted HR=2.01, 95% CI 1.31 to 3.07, P=1.20×10^{-3}) than for men (adjusted HR=1.25, 95% CI 1.01 to 1.54, P=0.039), (Table 6). OSA did not, however, associate with stroke risk (adjusted HR=0.99, 95% CI 0.75 to 1.33, P=0.98).

5.2.2 The effect of obstructive sleep apnoea on type 2 diabetes and its complications

The role of OSA as a risk factor for T2D was evaluated, showing an association with increased risk among individuals with OSA (HR=2.52, 95% CI 2.16 to 2.93, P=1.91×10^{-32}). The risk was somewhat attenuated after further adjustment for BMI (adjusted HR=1.48, 95% CI 1.26 to 1.73, P=9.11×10^{-7}) revealing a corresponding effect in both cohorts. Similarly, the association was more notable in women (adjusted HR=1.63, 95% CI 1.20 to 2.23, P=2.20×10^{-3}) than in men (adjusted HR =1.44, 95% CI 1.27 to 2.21, P=9.62×10^{-5}), (Table 7).

Table 7. Association between obstructive sleep apnoea and the population for incident type 2 diabetes

	Number of events/ Subjects at risk	Unadjusted model		Adjusted model	
All		HR [95% CI]	P	HR [95% CI]	P
FINRISK	2435/27161	2.40 [2.03-2.84]	1.53×10^{-24}	1.38 [1.16-1.64]	2.74×10^{-4}
H2000	455/6181	3.18 [2.20-4.59]	7.03×10^{-10}	2.05 [1.42-2.97]	1.41×10^{-4}
Combined	2890/33342	2.52 [2.16-2.93]	1.91×10^{-32}	1.48 [1.26-1.73]	9.11×10^{-7}
Men					
FINRISK	1372/12880	2.21 [1.81-2.69]	2.55×10^{-15}	1.28 [1.05-1.57]	0.017
H2000	257/2772	3.65 [2.44-5.44]	2.23×10^{-10}	2.27 [1.51-3.41]	8.08×10^{-5}
Combined	1629/15652	2.43 [2.04-2.90]	4.16×10^{-23}	1.44 [1.27-2.21]	9.62×10^{-5}
Women					
FINRISK	1063/14281	3.14 [2.28-4.33]	3.12×10^{-12}	1.65 [1.18-2.29]	2.98×10^{-3}
H2000	198/3409	2.16 [0.80-5.87]	0.13	1.48 [0.55-4.02]	0.44
Combined	1261/17690	3.03 [2.23-4.12]	1.25×10^{-15}	1.63 [1.20-2.23]	2.20×10^{-3}

The FINRISK unadjusted model is adjusted for age, cohort year, geographical area and gender. The adjusted model is adjusted for body mass index (BMI) in addition to covariates of the unadjusted model. The Health 2000 Survey (H2000) unadjusted model is adjusted for geographical area and gender. The adjusted model is adjusted for BMI in addition to covariates of the unadjusted model. HR=hazard ratio, CI=confidence interval. The table is adapted from the manuscript Strausz et al. (2018) *Obstructive sleep apnoea and the risk for coronary heart disease and type 2 diabetes: a longitudinal population-based study in Finland.*

The Botnia cohort was included in the meta-analysis to study T2D complications more in detail as H2000 lacked incident DKD events among patients with OSA. OSA associated with increased risk for DKD (HR=2.16, 95% CI 1.40 to 3.34, P=5.00×10^{-4}) among T2D patients. After adjusting for the known risk factors for DKD (BMI and hypertension), the estimate was slightly attenuated (adjusted HR=1.75, 95% CI 1.13 to 2.71, P=0.013). The effects were similar in both cohorts, (Table 8).

Table 8. Associations between obstructive sleep apnoea and incident type 2 diabetes complications

	Number of events/ Subjects at risk	Unadjusted model		Adjusted model	
DKD		HR [95% CI]	P	HR [95% CI]	P
FINRISK	147/3932	2.15 [1.27-3.62]	4.10×10^{-3}	1.72 [1.01-2.93]	0.044
Botnia	91/1380	2.19 [1.003-4.79]	0.049	1.80 [0.82-3.96]	0.143
Combined	238/5312	2.16 [1.40-3.34]	5.00×10^{-4}	1.75 [1.13-2.71]	0.013
CHD		HR [95% CI]	P	HR [95% CI]	P
FINRISK	640/3710	1.44 [1.07-1.95]	0.016	1.40 [1.04-1.90]	0.028
H2000	152/761	1.46 [0.74-2.82]	0.272	1.46 [0.74-2.89]	0.274
Botnia	236/1253	1.18 [0.60-2.31]	0.630	1.07 [0.54-2.11]	0.840
Combined	1028/5724	1.40 [1.10-1.81]	8.50×10^{-3}	1.36 [1.05-1.76]	0.019

The FINRISK unadjusted models are adjusted for age, cohort year, geographical area and gender. The Health 2000 Survey (H2000) unadjusted models are adjusted for age, geographical area and gender. The Botnia unadjusted models are adjusted for age and gender. The adjusted models for diabetic kidney disease (DKD) are adjusted for body mass index (BMI) and hypertension in all cohorts in addition to covariates of the unadjusted model. The FINRISK adjusted model for coronary heart disease (CHD) is adjusted for high density lipoprotein (HDL) and total cholesterol (CHOL), current cigarette smoking, BMI, hypertension and family history of stroke or myocardial infarction in addition to covariates of unadjusted model. The H2000 and Botnia adjusted models for CHD are adjusted for HDL and CHOL, current cigarette smoking, BMI and hypertension in addition to covariates of the unadjusted model. HR=hazard ratio, CI=confidence interval. The table is adapted from the manuscript Strausz et al. (2018) *Obstructive sleep apnoea and the risk for coronary heart disease and type 2 diabetes: a longitudinal population-based study in Finland.*

OSA alone was associated with an increased risk for CHD by 1.40-fold (95% CI 1.10 to 1.81, P=8.50×10^{-3}) among patients with T2D. The result was almost the same after adjusting for the following risk factors: HDL and total cholesterol, current cigarette smoking, BMI, hypertension and family history of stroke or myocardial infarction (adjusted HR=1.36, 95% CI 1.05 to 1.76, P=0.019), (Table 8).

5.2.3 The association between obstructive sleep apnoea and mortality risk

OSA's role as an independent risk factor for all-cause mortality was also investigated. OSA was associated with an increased risk for mortality (HR=1.18, 95% CI 1.00 to 1.40, P=0.057), but this risk was attenuated after adjusting for other confounding factors. Among T2D patients, OSA was related to increased mortality risk (HR=1.40, 95% CI 1.21 to 1.62, $P=2.03\times10^{-6}$). This estimate also remained significant after adjustments (HR=1.35, 95% CI 1.06 to 1.71, P=0.016), (Table 9).

Table 9. Associations between obstructive sleep apnoea and all-cause mortality among general population and type 2 diabetes (T2D) individuals

	Number of events/ Subjects at Risk	**Unadjusted model**		**Adjusted model**	
General population		HR [95% CI]	P	HR [95% CI]	P
FINRISK	3228/28666	1.08 [0.89-1.31]	0.438	1.01 [0.83-1.22]	0.949
H2000	1286/6498	1.65 [1.14-2.39]	7.91×10^{-3}	1.74 [1.20-2.52]	3.68×10^{-3}
Combined	4514/35164	1.18 [1.00-1.40]	0.057	1.13 [0.95-1.34]	0.161
T2D		HR [95% CI]	P	HR [95% CI]	P
FINRISK	719/3940	1.37 [1.01-1.84]	0.041	1.23 [0.91-1.67]	0.179
H2000	284/820	1.35 [0.68-2.71]	0.390	1.48 [0.74-2.98]	0.267
Botnia	348/1309	1.84 [1.14-2.99]	1.44×10^{-4}	1.62 [1.00-2.65]	0.052
Combined	1351/6069	1.40 [1.21-1.62]	2.03×10^{-6}	1.35 [1.06-1.71]	0.016

The FINRISK unadjusted models are adjusted for age, cohort year, geographical area and gender. The Health 2000 Survey (H2000) unadjusted models are adjusted for age, geographical area and gender. The Botnia unadjusted models are adjusted for age and gender. The FINRISK adjusted model is adjusted for high density lipoprotein (HDL) and total cholesterol (CHOL), current cigarette smoking, body mass index (BMI), hypertension and family history of stroke or myocardial infarction in addition to covariates of unadjusted model. The H2000 and Botnia adjusted models are adjusted for HDL, CHOL, current cigarette smoking, BMI and hypertension in addition to covariates of the unadjusted model. Adjusted models for general population are also adjusted for prevalent type 2 diabetes. HR=hazard ratio, CI=confidence interval. The table is adapted from the manuscript Strausz et al. (2018) *Obstructive sleep apnoea and the risk for coronary heart disease and type 2 diabetes: a longitudinal population-based study in Finland.*

OSA was associated with increased risk for all-cause mortality among patients with T2D but was not significantly associated with increased risk for all-cause mortality in the general population. The main cause of mortality was CHD both in T2D patients (33.8%) and in the general population (30.8%).

5.3 Genetic studies

To estimate the strengths of genetic associations between OSA and OSA-related comorbidities, data from 217,955 individuals who participated in the FinnGen project were utilized. 16,761 (7.7%) had an OSA diagnosis and 10,557 (63%) cases were male. The diagnoses were derived from ICD-codes in the Finnish National Hospital Discharge Registry and from the Causes of Death Registry. Baseline characteristics of the FinnGen participants and odds ratios (ORs) for OSA-associated comorbidities are presented in Table 10.

Table 10. Baseline characteristics and previously known obstructive sleep apnoea (OSA) comorbidities in OSA and non-OSA individuals

	All	Non-OSA	OSA	OR [95% CI]	P
	N=217955	N=201194	N=16761		
Male (N, %)	94799 (43.5)	84242 (41.9)	10557 (63.0)	2.26 [2.19-2.34]	$< 2.00 \times 10^{-16}$
Female (N, %)	123156 (56.5)	116952 (58.1)	6204 (37.0)		
Age (mean in years, SD)	52.4 (17.5)	51.8 (17.7)	58.9 (13.3)	1.02 [1.02-1.03]	$< 2.00 \times 10^{-16}$
Age at OSA diagnosis (mean, SD)			55.3 (11.9)		
BMI (mean kg/m^2, SD)	27.25 (5.34)	26.87 (5.02)	31.72 (6.74)	1.15 [1.15-1.16]	$< 2.00 \times 10^{-16}$
Hypertension (N, %)	55678 (25.5)	47549 (23.6)	8129 (48.5)	2.44 [2.36-2.53]	$< 2.00 \times 10^{-16}$
T2D (N, %)	29054 (13.3)	23932 (11.9)	5122 (30.6)	2.60 [2.50-2.70]	$< 2.00 \times 10^{-16}$
CHD (N, %)	20925 (9.6)	18495 (9.2)	2430 (14.5)	1.11 [1.06-1.17]	1.04×10^{-5}
Stroke (N, %)	11671 (5.4)	10414 (5.2)	1257 (7.5)	1.10 [1.03-1.17]	3.29×10^{-3}
Depression (N, %)	23160 (10.6)	20094 (10.0)	3066 (18.3)	2.56 [2.45-2.67]	$< 2.00 \times 10^{-16}$
Hypothyroidism (N, %)	26228 (12.0)	23384 (11.6)	2844 (17.0)	1.85 [1.77-1.94]	$< 2.00 \times 10^{-16}$
Asthma (N, %)	20520 (9.4)	17358 (8.6)	3162 (18.9)	2.58 [2.47-2.69]	$< 2.00 \times 10^{-16}$
IRD (N, %)	12961 (5.9)	11555 (5.7)	1406 (8.4)	1.48 [1.39-1.57]	$< 2.00 \times 10^{-16}$

Age and body mass index (BMI) were measured at the time that the biobank sample was given. BMI was measured for 159,731 individuals including 12,759 OSA cases and 146,972 controls. T2D=type 2 diabetes, CHD=coronary heart disease, IRD=inflammatory rheumatic diseases, OR=odds ratio, CI=confidence interval, SD=standard deviation. The table is adapted from the manuscript Strausz et al. (2018) *Obstructive sleep apnoea and the risk for coronary heart disease and type 2 diabetes: a longitudinal population-based study in Finland.*

5.3.1 Genome-wide findings and heritability

In the GWAS, five novel genetic loci were found to associate with OSA ($P<5.0\times10^{-8}$), (Table 11, Figure 7a). In addition, the heritability of OSA in the FinnGen study was estimated to be 0.08 (95% CI 0.06 to 0.11) before and 0.06 (95% CI 0.04 to 0.08) after adjustment for BMI.

The lead variant in chromosome 16 was rs9937053, an intronic variant in the gene Fat mass and obesity-associated protein (*FTO)*, $P=4.3\times10^{-16}$. In chromosome 12, the lead variant was rs10507084, near Rhabdomyosarcoma 2 associated transcript *(RMST)/* NEDD1 gamma-tubulin ring complex targeting factor *(NEDD1)*, $P=2.8\times10^{-11}$. *RMST*, a long non-coding RNA, was the nearest gene and *NEDD1* the nearest protein coding gene. On chromosome 10, the lead variant was rs185932673, an intronic variant in Calcium/calmodulin-dependent protein kinase 1D *(CAMK1D),* $P=2.4\times10^{-8}$. In chromosome 9, the lead variant was rs4837016 near GTPase activating protein and VPS9 domains 1 (*GAPVD1)*, $P=1.5\times10^{-8}$ and in chromosome 2, the lead variant rs10928560 was near C-X-C motif chemokine receptor 4 (*CXCR4)*, $P=2.8\times10^{-8}$. Four out of five of these OSA-associated lead variants have also been previously associated with BMI ($P<0.01$) (214-216), with the exception of rs10507084 at the *RMST/NEDD1* locus. Conditional analyses of the associated loci did not suggest any additional associations.

Adjusting for BMI did not affect the association for variant rs10507084 (Table 11, Figure 7b), ($OR_{unadjusted}=1.11$, 95% CI 1.08 to 1.15, $P=2.8\times10^{-11}$ vs. $OR_{BMI\ adjusted}=1.12$, 95% CI 1.08 to 1.17, $P=9.7\times10^{-11}$) suggesting a BMI-independent mechanism for rs10507084 in OSA predisposition. As a sensitivity analysis we conducted a GWAS where individuals with snoring (ICD-10: R06.5) were removed, after which 197,797 individuals remained in the control group. No new associations were observed, and it did not notably affect our estimates.

Table 11. Characterization of five genome-wide significant obstructive sleep apnoea loci

CHR	Position	RSID	REF	ALT	Nearest gene	Consequence	Fin. enr.	AF	AF cases	AF controls	INFO	OR [95% CI]	P	OR [95%CI] BMIadj	P BMIadj
16	53765595	rs9937053	G	A	FTO	intron	0.97	0.43	0.45	0.43	0.999	1.11[1.08-1.13]	4.3×10^{-16}	1.03[1.001.06]	0.04
12	97359374	rs10507084	C	T	RMST/ NEDD1	intergenic	3.03	0.18	0.19	0.18	0.993	1.11[1.08-1.15]	2.8×10^{-11}	1.12[1.08-1.17]	9.7×10^{-10}
10	12656440	rs185932673	C	T	CAMK1D	intron	0.55	0.0033	0.0051	0.0032	0.972	1.87[1.50-2.33]	2.4×10^{-8}	1.75[1.37-2.26]	9.3×10^{-6}
9	125379530	rs4837016	G	A	GAPVD1	intergenic	1.12	0.47	0.45	0.47	0.995	0.93[0.91-0.95]	1.5×10^{-8}	0.95[0.92-0.97]	2.2×10^{-4}
2	136234237	rs10928560	C	T	CXCR4	downstream	1.04	0.20	0.18	0.20	0.993	0.92[0.89-0.94]	2.8×10^{-8}	0.93[0.900.96]	8.5×10^{-5}

All effect sizes and allele frequencies are reported in terms of alternate allele (ALT). The finding of rs185932673 should be interpreted cautiously, as the variant is rare in the Finnish population. CHR=chromosome, REF=reference allele, Fin.enr=Finnish enrichment is computed using the Genome Aggregation Database (gnomAD) data comparing Finnish to other European populations, AF=allele frequency, INFO= imputation quality score, OR=odds ratio, CI=confidence interval, P-value BMIadj=P-value after body mass index (BMI) adjustment. *FTO=Fat mass and obesity-associated protein, RMST=Rhabdomyosarcoma 2 associated transcript / NEDD1=NEDD1 gamma-tubulin ring complex targeting factor, CAMK1D=Calcium/calmodulin-dependent protein kinase 1D, GAPVD1=GTPase activating protein and VPS9 domains 1, CXCR4=C-X-C motif chemokine receptor 4.* The table is adapted from the manuscript Strausz et al. (2020) *Genetic analysis of obstructive sleep apnoea discovers a strong association with cardiometabolic health.*

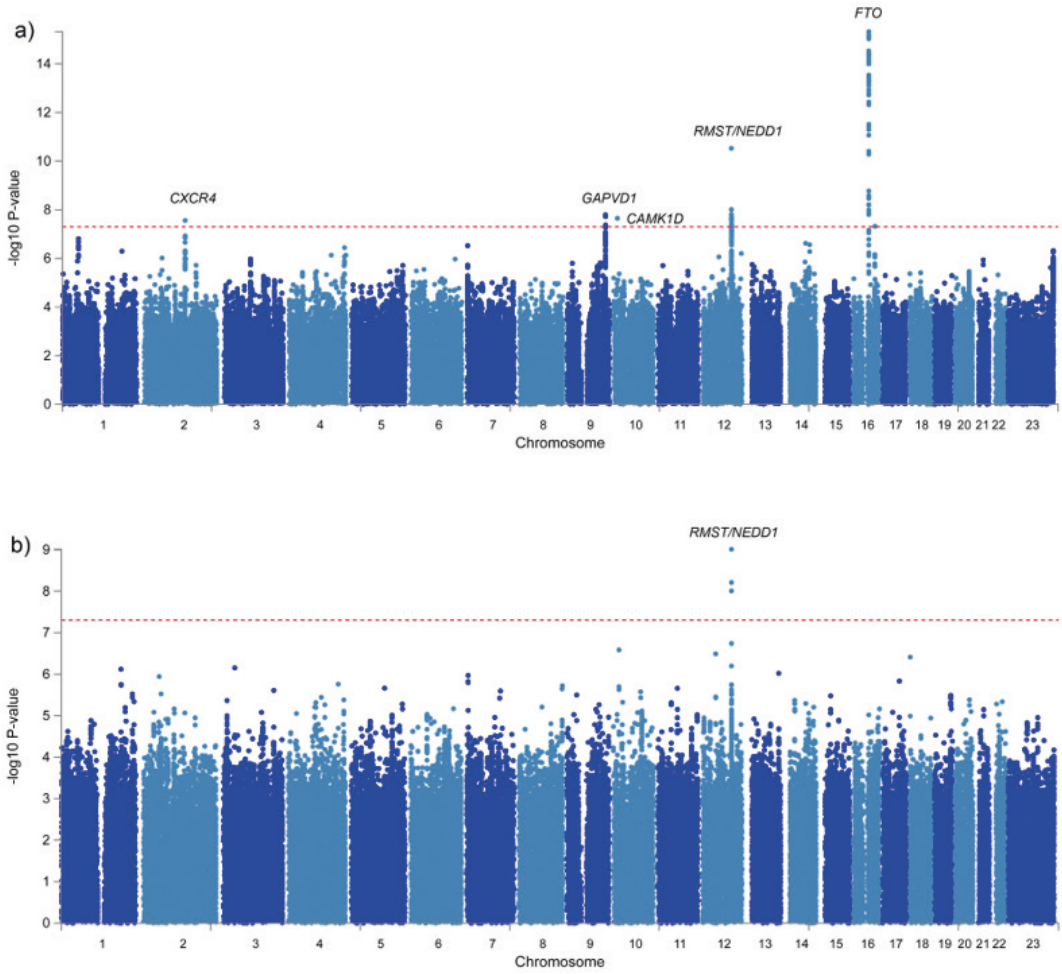

Figure 7. a) Manhattan plot for the genome-wide association study (GWAS) of obstructive sleep apnoea (OSA) including 16,761 OSA cases and 201,194 controls. For each genetic variant, the x-axis shows chromosomal position, while y-axis shows the $-\log_{10}(P)$ value. The horizontal line indicates the genome-wide significance threshold of $P=5\times10^{-8}$. Five genetic loci were identified at a genome-wide significant level. *CXCR4*=C-X-C motif chemokine receptor 4, *GAPVD1*=GTPase activating protein and VPS9 domains 1, *CAMK1D*=Calcium/calmodulin-dependent protein kinase 1D, *RMST*=Rhabdomyosarcoma 2 associated transcript / *NEDD1*=NEDD1 gamma-tubulin ring complex targeting factor, *FTO*=Fat mass and obesity-associated protein.

b) Manhattan plot for the GWAS of OSA after body mass index (BMI) adjustment including 12,759 OSA cases and 146,972 controls. For each genetic variant, the x-axis shows chromosomal position, while y-axis shows the $-\log_{10}(P)$ value. The horizontal line indicates the genome-wide significance threshold of $P=5\times10^{-8}$. One genetic locus was identified at a genome-wide significant level. The figure is adapted from the manuscript Strausz et al. (2020) *Genetic analysis of obstructive sleep apnoea discovers a strong association with cardiometabolic health.*

5.3.1.1 Replication

To replicate the lead variants associated with OSA, additional and comparable cohorts (UKBB, ANDIS and ESTBB) were used. The results were pooled using inverse-variance weighted fixed effect meta-analysis. These independent datasets supported the role of the *FTO* and *GAPVD1* loci in OSA (P<0.05), (Table 12).

Table 12. Replication of the lead variants

CHR	RSID	G47.3 OSA UKBB	G47.3 OSA ANDIS	G47.3 OSA ESTBB	G47.3 OSA Combined
		N cases: 4471 N controls: 403723	N cases: 947 N controls: 9829	N cases: 4930 N controls: 61056	N cases: 10348 N controls: 474608
16	rs9937053	OR=1.12 [1.07-1.17] P=5.5×10^{-7}	OR=1.13 [1.03-1.24] P=0.01	OR=1.06 [1.02-1.11] P=6.6×10^{-3}	OR=1.09 [1.06-1.12] P=2.7×10^{-9}
12	rs10507084	OR=1.07 [0.98-1.17] P=0.15	OR=0.89 [0.73-1.06] P=0.18	OR=1.01 [0.94-1.09] P=0.80	OR=1.02 [0.96-1.08] P=0.51
10	rs185932673	OR=0.96 [0.73-1.26] P=0.74	Not defined in ANDIS	OR=1.09 [0.84-1.43] P=0.52	OR=1.02 [0.84-1.23] P=0.82
9	rs4837016	OR=0.97 [0.93-1.01] P=0.16	OR=0.87 [0.79-0.95] P=4.6×10^{-3}	OR=0.98 [0.94-1.02] P=0.32	OR=0.96 [0.94-0.99] P=0.01
2	rs10928560	OR=1.00 [0.94-1.06] P=0.94	OR=1.01 [0.90-1.15] P=0.38	OR=1.01 [0.96-1.07] P=0.60	OR=1.01 [0.97-1.05] P= 0.57

Inverse-variance weighted meta-analysis combining the results of the replication cohorts of the main FinnGen findings considering obstructive sleep apnoea (OSA). CHR=chromosome, OR=odds ratio, [95% confidence interval], UKBB = UK Biobank, ANDIS = All New Diabetics in Scania, ESTBB = Estonian Biobank. The table is adapted from the manuscript Strausz et al. (2020) *Genetic analysis of obstructive sleep apnoea discovers a strong association with cardiometabolic health.*

PRSes were calculated using the lead variants from our study to predict OSA in the UKBB's individual-level data. This revealed an association with OSA risk, where the individuals in the highest OSA PRS quintile had a modest 1.24-fold (95% CI 1.15 to 1.33, P=6.89×10^{-9}) increased OSA risk compared with the lowest quintile after adjusting for birth year, sex and the 10 first PCs. After the association was adjusted for BMI (in addition to the aforementioned covariates), a somewhat lower but still significant estimate was still found (OR=1.11, 95% CI 1.03 to 1.20, P=4.70×10^{-3}), (Table 13).

Table 13. Association between polygenic risk score (PRS) and obstructive sleep apnoea (OSA) in the UK Biobank

	Model 1			Model 2		
	OR	95% CI	P	OR	95% CI	P
OSA_Q1	Reference			Reference		
OSA_Q2	1.07	0.92-1.15	0.080	1.02	0.95-1.10	0.585
OSA_Q3	1.09	1.01-1.18	0.029	1.03	0.95-1.11	0.464
OSA_Q4	1.10	1.02-1.19	0.013	1.00	0.92-1.08	0.966
OSA_Q5	1.24	1.15-1.33	6.89×10^{-9}	1.11	1.03-1.20	4.70×10^{-3}

Model 1 is adjusted for age, sex and the 10 first principal components. Model 2 is adjusted for body mass index in addition to the covariates of Model 1. The OSA PRS was stratified into quintiles and OSA_Q5 is the highest quintile. OR=odds ratio, CI = 95% confidence interval. The table is adapted from the manuscript Strausz et al. (2020) *Genetic analysis of obstructive sleep apnoea discovers a strong association with cardiometabolic health.*

5.3.2 Gene based analyses

MAGMA was used in an exploratory analysis to annotate FinnGen OSA summary statistics based on 18 biological data repositories and tools (213). 25 significant associations ($P<2.54\times10^{-6}$) with various biological processes were detected, which were driven by the same loci as the significant GWAS variants in *FTO* and *GAPVD1* (Figure 8a).

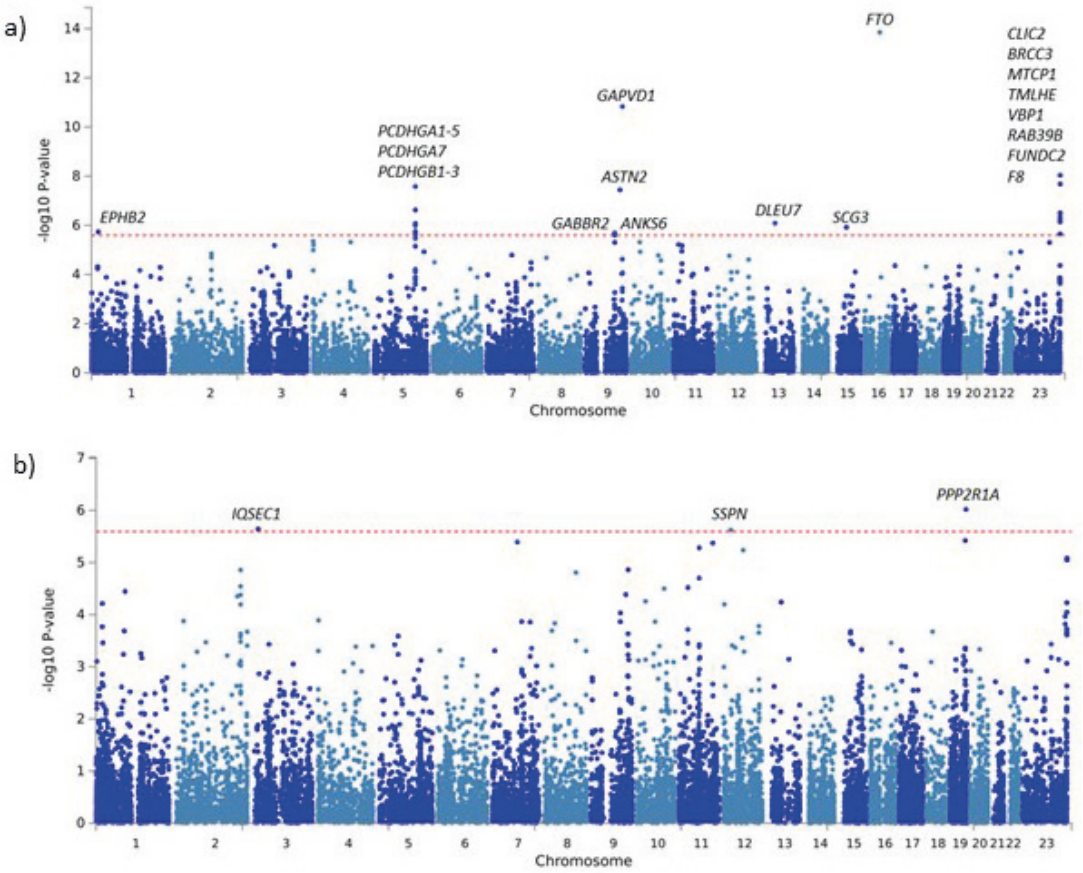

Figure 8. a) Manhattan plot of the gene-based test as computed by multi-marker analysis of genomic annotation (MAGMA) using the OSA genome-wide association study (GWAS) data. Single nucleotide polymorphisms (SNPs) were mapped to 19,651 protein coding genes. A Bonferroni-corrected significance threshold was defined as P =0.05/19,651=2.54×10^{-6}. Primarily the same genes were identified as in the single variant associations. For each annotated gene, the x-axis shows the chromosomal position while the y-axis shows the −log10(P) value. *EPHB2*=Ephrin type-B receptor 2, *PCDHGA*=Protocadherin gamma subfamily A, *PCDHGB*=Protocadherin gamma subfamily B, *GAPVD1*=GTPase activating protein and VPS9 domains 1, *ASTN2*= Astrotactin 2, *GABBR2*=Gamma-aminobutyric acid type A receptor subunit 2, *ANKS6*=Ankyrin repeat and sterile alpha motif domain containing 6, *DLEU7*=Deleted in lymphocytic leukaemia 7, *SCG3*=Secretogranin III, *FTO*=Fat mass and obesity-associated protein, *CLIC2*=Chloride intracellular channel 2, *BRCC3*=BRCA1/BRCA2-containing complex subunit 3, *MTCP1*=Mature T cell proliferation 1, *TMLHE*=Trimethyllysine hydroxylase, epsilon, *VBP1*= VHL binding protein 1, *RAB39B*=RAB39B, member RAS oncogene family, *FUNDC2*=FUN14 domain containing 2 and *F8*= Coagulation factor VIII.

b) Manhattan plot of the gene-based test as computed by MAGMA using body mass index (BMI)-adjusted GWAS data. SNPs were mapped to 19,651 protein coding genes. A Bonferroni-corrected significance threshold was defined as P=0.05/19,651=2.54×10^{-6}. For each annotated gene the x-axis shows the chromosomal position while the y-axis shows the −log10(P) value. *IQSEC1*= IQ motif and sec7 domain arfGEF 1, *SSPN*=Sarcospan, *PPP2R1A*= Protein phosphatase 2 scaffold subunit aalpha. The figure is adapted from the manuscript Strausz et al. (2020) *Genetic analysis of obstructive sleep apnoea discovers a strong association with cardiometabolic health*.

These may be potential target genes that associate with OSA, indicating that the genes could be relevant for OSA or OSA-associated comorbidities. The gene-based test for BMI-adjusted OSA also revealed three further associated genes (Figure 8b).

5.3.3 Phenome-wide findings

A PheWAS was performed using FinnGen data to study the associations between the lead SNPs and 2,925 disease endpoints. Rs10507084 was again specific for OSA after adjustment for BMI. This suggests an association between rs10507084 and OSA independent of cardiometabolic traits (Figure 9a). *FTO* was associated with OSA, but also with a wide spectrum of cardiometabolic diagnoses as shown earlier (9, 214) and also to coffee consumption (217). The strongest PheWAS associations were observed with OSA-related diseases such as obesity ($P=4.14\times10^{-41}$), T2D ($P=5.67\times10^{-28}$) and hypertension ($P=1.40\times10^{-10}$). In addition, there was a strong correlation between rs10507084 and the use of antidepressants (OR=1.013, 95% CI 1.007 to 1.019, $P=4.4\times10^{-6}$) (Figure 9b). This result remained significant after further adjustment for OSA diagnosis (OR=1.011, 95% CI 1.005 to 1.017, $P=1.9\times10^{-4}$).

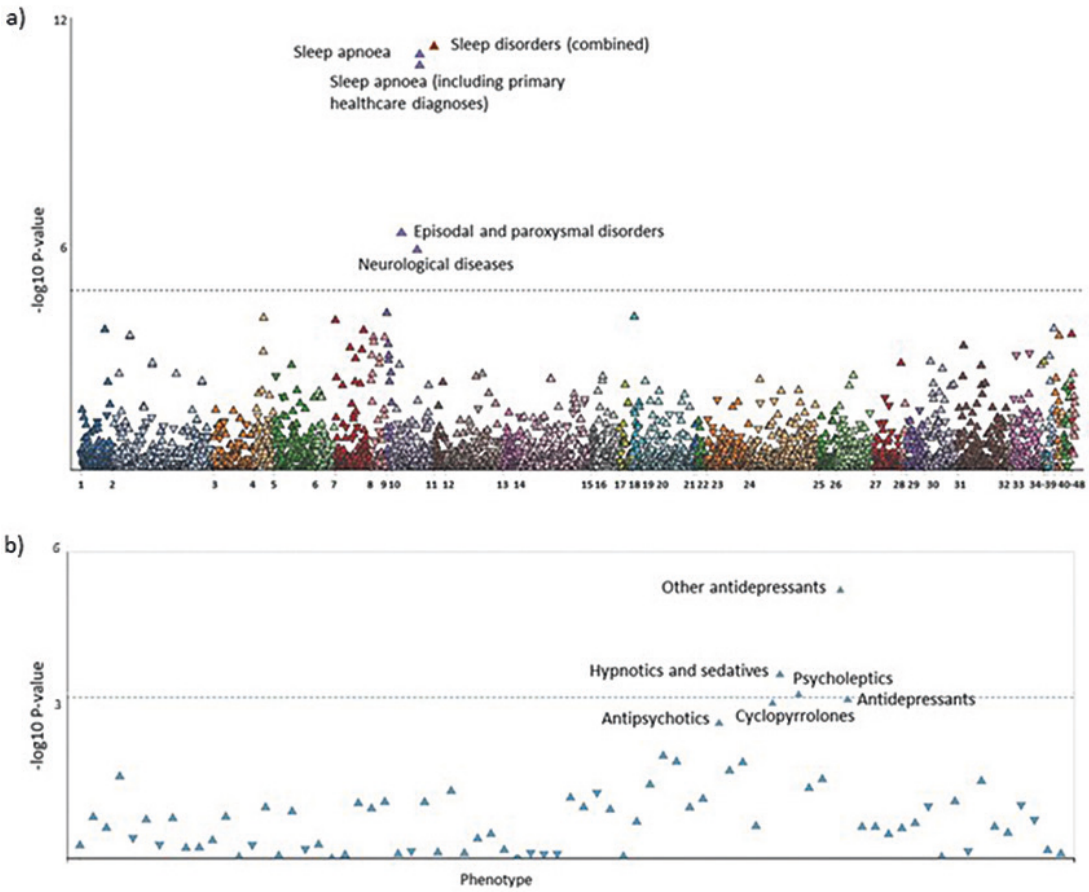

Figure 9. a) Phenome-wide association study (PheWAS) associations after body mass index (BMI) adjustment between rs10507084 and 2,925 disease endpoints. A Bonferroni-corrected significance threshold was defined as P = 0.05/2925 = 1.71×10^{-5}. The vertical axis displays association P-values on the $-\log_{10}$ scale. Sleep apnoea refers to the validated disease endpoint. Primary health care diagnoses have not been validated. Sleep disorders, Episodal and paroxysmal disorders and Neurological diseases include sleep apnoea. The disease definition along the horizontal axis: 1. Certain infectious and parasitic diseases, 2. Neoplasms from hospital discharges , 3. Neoplasms, from cancer registry , 4. Diseases of the blood and blood-forming organs and certain disorders involving the immune mechanism, 5. Endocrine, nutritional and metabolic diseases, 6. Diabetes endpoints, 7. Mental and behavioural disorders, 8. Psychiatric endpoints, 9. Alcohol related diseases, 10. Diseases of the nervous system, 11. Neurological endpoints, 12. Diseases of the eye and adnexa, 13. Diseases of the ear and mastoid process, 14. Diseases of the circulatory system, 15. Cardiometabolic endpoints, 16. Diseases of the respiratory system, 17. Asthma and related endpoints, 18. Chronic obstructive pulmonary disease and related endpoints, 19. Interstitial lung disease endpoints, 20. Diseases of the digestive system, 21. Dental endpoints, 22. Gastrointestinal endpoints, 23. Diseases of the skin and subcutaneous tissue, 24. Diseases of the musculoskeletal system and connective tissue, 25. Rheumatoid arthritis endpoints, 26. Diseases of the genitourinary system, 27. Pregnancy, childbirth and the puerperium , 28. Certain conditions originating in the perinatal period, 29. Congenital malformations, deformations and chromosomal abnormalities, 30. Symptoms, signs and abnormal clinical and laboratory findings, not elsewhere classified, 31. Injury, poisoning and certain other consequences of external causes, 32. External causes of morbidity and mortality, 33. Factors influencing health status and contact with health services, 34. Drug purchase endpoints, 35. Diseases marked as autoimmune origin,

36. Common endpoint, 37. Demonstration endpoints, 38. ICD-10 main chapters, 39. Operation endpoints, 40. Other, not yet classified endpoints, 41. Miscellaneous, not yet classified endpoints, 42. Comorbidities of Asthma, 43. Comorbidities of Chronic obstructive pulmonary disease, 44. Comorbidities of Diabetes, 45. Comorbidities of Gastrointestinal endpoints, 46. Comorbidities of Interstitial lung disease endpoints, 47. Comorbidities of Neurological endpoints, 48. Comorbidities of Rheumatoid arthritis endpoints

b) PheWAS analysis concerning drug purchases. The x-axis shows phenotypes based on Anatomical Therapeutic Chemical System (ATC) drug codes, while the y-axis shows the Bonferroni-corrected significance threshold ($0.05/69 = 7.25 \times 10^{-4}$) $-\log_{10}(P)$ values. Drugs were coded as continuous variables and inverse normalized to ensure normal distribution for analysis. Other antidepressant=ATC N06AX, Hypnotics and sedatives=ATC N05C, Psycholeptics=ATC N05, Antidepressants=ATC N06A, Cycclopyrrolones=ATC N05CF, Antipsychotics=ATC N05A. The figure is adapted from the manuscript Strausz et al. (2020) *Genetic analysis of obstructive sleep apnoea discovers a strong association with cardiometabolic health.*

An association near *RMST/NEDD1* was specific for OSA independent of BMI. The lead SNP was associated with antidepressant purchases, which connects this locus with the regulation of sleep and mood. The finding may also reflect the earlier observation that depression is prevalent among patients with OSA (100).

5.3.4 Genetic correlations and Mendelian randomization

The potential genetic associations between OSA and its known epidemiological correlates were studied using FinnGen summary statistics to calculate genetic correlations. Strong genetic correlations between OSA and BMI (r_g=0.72, 95% CI 0.62 to 0.83, P=3.49×10^{-40}) and between OSA and other OSA-related comorbidities were found: hypertension (r_g=0.35, 95% CI 0.23 to 0.48, P=4.06×10^{-8}), T2D (r_g=0.52. 95% CI 0.37 to 0.66, P=6.40×10^{-12}), CHD (r_g=0.38, 95% CI 0.17 to 0.58, P=3.84×10^{-4}), stroke (r_g=0.33, 95% CI 0.03 to 0.63, P=2.93×10^{-2}), depression (r_g=0.43, 95% CI 0.27 to 0.60, P=2.79×10^{-7}), hypothyroidism (r_g=0.40, 95% CI 0.27 to 0.54, P=7.07×10^{-9}), asthma (r_g=0.50, 95% CI 0.33 to 0.68, P=1.53×10^{-8}) and IRD (r_g=0.34, 95% CI 0.09 to 0.58, P=6.97×10^{-3}). Additionally, high genetic correlations between OSA-related comorbidities were observed. Since many OSA comorbidities are associated with BMI, the genetic correlations were then calculated after BMI adjustment. This analysis showed somewhat lower estimates for genetic correlations between OSA and CHD (r_g=0.24 95% CI 0.012 to 0.47, P=0.04), depression (r_g=0.33, 95% CI 0.17 to 0.50, P=1.1×10^{-3}), asthma (r_g=0.33, 95% CI 0.11 to 0.54, P=2.6×10^{-3}) and hypothyroidism, (r_g=0.28, 95% CI 0.11 to 0.44, P=8.0×10^{-4}). Genetic correlations between OSA and BMI (r_g=0.08, 95% CI -0.05 to 0.22, P=0.22), hypertension (r_g=0.05, 95% CI -0.10 to 0.20, P=0.51), T2D (r_g=0.15, 95% CI -0.03 to 0.33, P=0.11), stroke (r_g=0.32, 95% CI -0.05 to 0.69, P=0.09) and IRD (r_g=0.27, 95% CI -0.01 to 0.54, P=5.7×10^{-2}) were attenuated after BMI adjustment (Figure 10). The estimate showed a strong positive genetic correlation between males and females (r_g=1, 95% CI 1.15 to 0.85), P=1.85×10^{-12}).

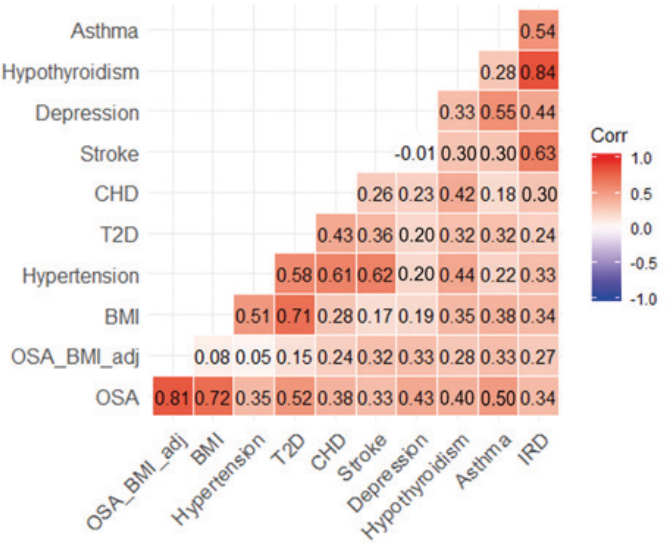

Figure 10. Genetic correlations between obstructive sleep apnoea (OSA), body mass index (BMI) and previously known OSA-related comorbidities using linkage disequilibrium score regression. The colour-scale represents the strength of the correlation. Correlations between OSA and other traits have been calculated with and without BMI-adjustment. CHD=coronary heart disease, T2D=type 2 diabetes, IRD=inflammatory rheumatic diseases. The figure is reproduced from the manuscript Strausz et al. (2020) *Genetic analysis of obstructive sleep apnoea discovers a strong association with cardiometabolic health.*

UKBB-derived summary statistics for sleep traits and the FinnGen OSA summary statistics were used to estimate genetic correlations of sleep traits. Genetic correlations were observed with snoring (207) (r_g=0.81, 95% CI 0.69 to 0.93, P=1.24×10^{-38}), sleep efficiency (210) =-0.31, 95% CI -0.44 to -0.17, P=9.80×10^{-6}) and daytime sleepiness (208) (r_g =0.44, 95% CI 0.33 to 0.54, P=1.27×10^{-15}). These associations remained significant also after BMI adjustment (r_g =0.68, 95% CI 0.55 to 0.81, P=2.93×10^{-26}, r_g =-0.19, 95% CI -0.36 to -0.03, P=0.02, r_g =0.42, 95% CI 0.29 to 0.55, P=1.06×10^{-10}, respectively). No significant genetic correlations between OSA and sleep duration or chronotype were noted (209), (Table 14).

The causal relationship between OSA and its comorbidities was tested by analysing OSA PRSes followed by MR analysis using FinnGen OSA summary statistics and independent BMI SNPs (9). A strong association with OSA risk was found and the individuals in the highest BMI PRS quintile had 1.98-fold increased (95% CI 1.88 to 2.09, P=3.38×10^{-140}) OSA risk after adjusting for age, sex and the 10 first PCs. This association was further confirmed in formal MR analysis. Sixty-four independent BMI SNPs (9) were used as genetic instruments to predict OSA. In line with former epidemiological studies and the genetic correlation results, a strong causal effect from BMI to OSA was discovered (Inverse variance weighted beta=0.67, P=8.32×10^{-16}), (Figure 11).

Table 14. Genetic correlations between obstructive sleep apnoea (OSA) and other sleep traits

	Snoring	Sleepiness	Sleep duration	Chronotype	Sleep efficiency
OSA	r_g = 0.81 [0.69-0.93] P = 1.24×10^{-38}	r_g = 0.44 [0.33-0.54] P = 1.27×10^{-15}	r_g = 0.01 [-0.09-0.10] P = 0.84	r_g = -5.0×10^{-4} [-0.08-0.08] P = 0.99	r_g = -0.31 [-0.44 - -0.17] P = 9.80×10^{-6}
OSA BMI-adjusted	*r_g = 0.68 [0.55-0.81) P = 2.93×10^{-26}	r_g = 0.42 [0.29-0.55] P = 1.06×10^{-10}	r_g = 0.08 [-0.03-0.19] P = 0.14	r_g = -0.06 [-0.15-0.03] P = 0.18	r_g = -0.19 [-0.36 - -0.03] P = 0.02

Summary statistics for sleep traits that were used to calculate the genetic correlations were obtained from previous genome-wide association studies (GWASes) from the UK Biobank. *GWAS for snoring was also body mass index (BMI)-adjusted. [95% confidence interval]. The table is adapted from the manuscript Strausz et al. (2020) *Genetic analysis of obstructive sleep apnoea discovers a strong association with cardiometabolic health.*

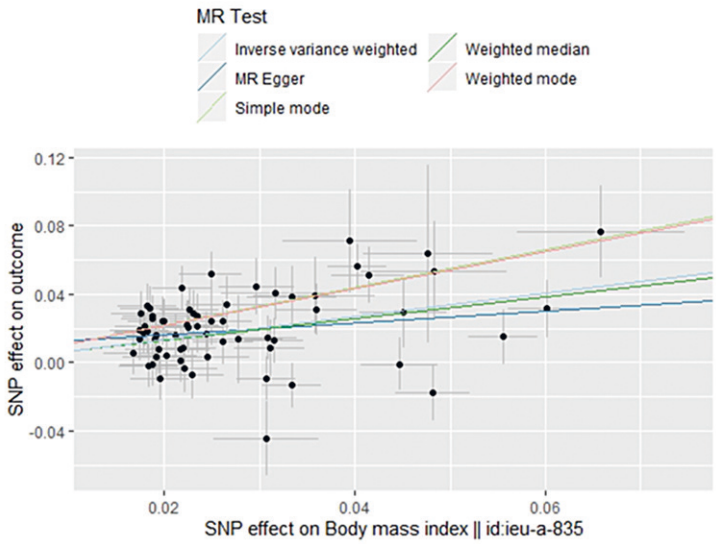

Figure 11. Formal Mendelian randomization (MR) analysis suggesting a strong causal relationship between body mass index (BMI) and obstructive sleep apnoea (OSA) where BMI predicts OSA as an outcome. The figure is reproduced from the manuscript Strausz et al. (2020) *Genetic analysis of obstructive sleep apnoea discovers a strong association with cardiometabolic health.*

Biological mechanisms of OSA were also examined through partitioned tissue enrichment analysis using LDSC, which combines data from the ENCODE and GTEx resources (211, 212) with FinnGen OSA summary statistics. The strongest association was with cardiovascular tissues and connective and bone tissues (P<0.05). Furthermore, enrichment in BMI-adjusted OSA results suggested the central nervous system as the strongest single-tissue association when a nominal significance level of P=0.05 is used (Figure 12).

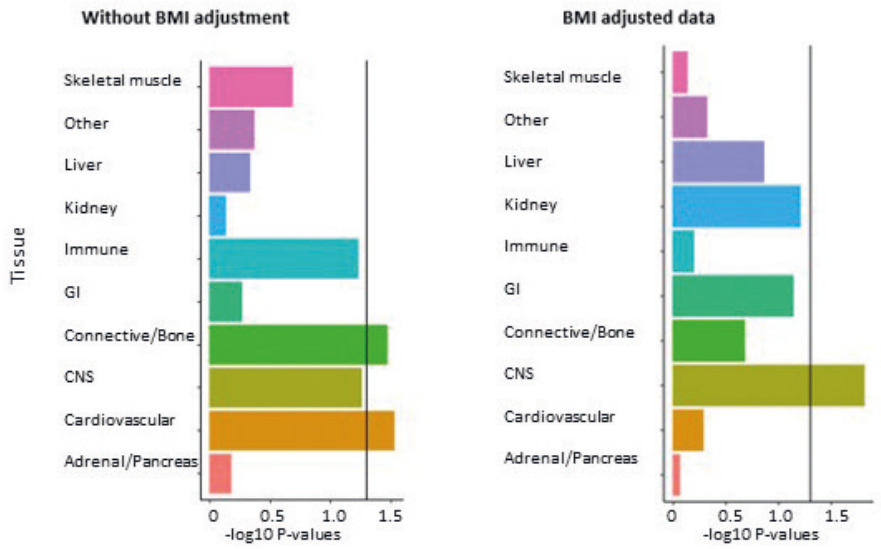

Figure 12. Tissue-specific enrichment analysis. Stratified linkage disequilibrium (LD) score regression was performed based on the 1000 Genomes Project phase 1 single nucleotide polymorphisms. LD was calculated for each tissue type. Each bar represents the -log10 P-value for enrichment and was computed for obstructive sleep apnoea (OSA) and body mass index (BMI)-adjusted OSA. CNS=central nervous system, GI=gastrointestinal. The figure is adapted from the manuscript Strausz et al. (2020) *Genetic analysis of obstructive sleep apnoea discovers a strong association with cardiometabolic health.*

5.4 Obstructive sleep apnoea as a risk factor for COVID-19

5.4.1 Findings in FinnGen

Mild OSA cases are not usually referred to secondary health care. Therefore, in our data the majority of the OSA individuals have a moderate or severe form of the disease. Our study also included 445 individuals with a PCR-validated COVID-19 diagnosis (37.3% male, mean age 52.7 years). Of these individuals, thirty-eight patients also had an OSA diagnosis (8.5%, 50.0% male, mean age 61.3 years), (Table 15, Table 16). The prevalence of OSA among COVID-19 patients is similar to the normal population in FinnGen, where the prevalence is 7.7%. Altogether, 91 (20.4%) patients required hospitalisation (36.3% male, mean age 65.9 years) including nineteen OSA patients (Table 15).

Table 15. Comparison of the baseline characteristics in hospitalised and non-hospitalised COVID-19 patients

	All N=445	Non-hospitalised N=354	Hospitalised N=91	$P_{unadjusted}$
Age (mean in years, SD)	52.7 (17.4)	49.3 (16.3)	65.9 (14.8)	1.06×10^{-15}
Sex (male) (N, %)	166 (37.3)	133 (37.6)	33 (36.3)	1
OSA (N, %)	38 (8.5)	19 (5.4)	19 (20.9)	5.13×10^{-5}
BMI (mean kg/m^2, SD)	27.13 (5.44)	26.54 (5.2)	29.25 (5.8)	0.014
Hypertension (N, %)	79 (17.8)	40 (11.3)	39 (42.9)	5.03×10^{-11}
Diabetes (N, %)	46 (10.3)	23 (6.5)	23 (25.3)	3.45×10^{-6}
CHD (N, %)	21 (4.7)	9 (2.5)	12 (13.2)	5.20×10^{-4}
Asthma/COPD (N, %)	54 (12.1)	40 (11.3)	14 (15.4)	1

Differences and associations between non-hospitalised and hospitalised COVID-19 patients. Statistics for baseline demographics and clinical characteristics ($P_{unadjusted}$) were based on the X^2 test. For continuous variables, we used Student's t-tests. Body mass index (BMI) was measured for 264 participants, including 206 non-hospitalised and 58 hospitalised individuals. OSA=obstructive sleep apnoea, CHD=coronary heart disease, COPD=chronic obstructive pulmonary disease, SD=standard deviation. The table is adapted from Strausz et al. (2020) *Sleep apnoea is a risk factor for severe COVID-19.*

The prevalences of OSA ($P=5.13\times10^{-5}$), hypertension ($P=5.03\times10^{-11}$), diabetes ($P=3.45\times10^{-6}$) and CHD ($p=5.20\times10^{-4}$) were higher in the hospitalised group. Similarly, age and BMI were higher among hospitalised individuals ($P=1.06\times10^{-15}$, P=0.014, respectively), (Table 15).

The main risk factors between COVID-19 patients with OSA (N=38) and those who did not have an OSA diagnosis (N=407) were compared. OSA patients were older, and their BMI was higher (mean age 61.3 years, BMI 31.15 kg/m^2, $P=5.60\times10^{-4}$, $P=3.38\times10^{-3}$, respectively) than non-OSA individuals (mean age 51.9 years, BMI 26.71 kg/m^2). Also, comorbidities were more prevalent among OSA individuals ($P=1.74\times10^{-4}$) and they faced hospitalisation more often ($P=3.21\times10^{-5}$). No differences were observed in risk factors when comparing non-hospitalised (N=19, male 63.2%, mean age 56.3 years) and hospitalised OSA patients (N=19, male 36.8%, mean age 66.3 years), (Table 16). In addition, seven out of nineteen patients with OSA who were hospitalised due to COVID-19 did not have any other disease comorbidities. No significant differences concerning age or BMI were noted between individuals who had only OSA as a comorbidity or also other comorbidities in addition to OSA (62.8 years, 68.9 years, P=0.72, 30.26 kg/m^2, 32.67 kg/m^2, P=1.00, respectively).

The time from first OSA diagnosis to COVID-19 infection was not significantly different between non-hospitalised and hospitalised groups (6.6 years, 8.6 years, P=0.37, respectively).

Table 16. Description of COVID-19 patients with or without obstructive sleep apnoea (OSA) and a comparison between non-hospitalised and hospitalised OSA patients

	Non-OSA N=407	**OSA N=38**	**P**	**OSA Non-hospitalised N=19**	**OSA hospitalised N=19**	**P**
Age (mean in years, SD)	51.9 (17.5)	61.3 (12.9)	5.60×10^{-4}	56.3 (11.0)	66.3 (12.9)	0.057
Sex (male) (N, %)	147 (36.1)	19 (50.0)	0.645	12 (63.2)	7 (36.8)	0.776
BMI (mean kg/m^2, SD)	26.71 (5.27)	31.15 (5.56)	3.38×10^{-3}	30.91 (5.58)	31.37 (5.77)	1
Comorbidities or outcomes (N, %)	118 (29.0)	24 (63.2)	1.74×10^{-4}	12 (63.2.)	12 (63.2)	1
Hospitalised (N, %)	72 (17.7)	19 (50.0)	3.21×10^{-5}			

Differences between non-OSA vs. OSA and non-hospitalised OSA patients vs. hospitalised OSA patients among COVID-19-diagnosed individuals. 7/19 patients who were hospitalised had an OSA diagnosis but did not have any other disease comorbidities. P-values were based on X^2 tests. Fisher's exact test was used if the sample size was ≤ 5. For continuous variables, we used Student's t-tests. BMI was measured for 264 participants including 239 non-OSA and 25 OSA individuals. BMI=body mass index, Comorbidities or outcomes=hypertension, diabetes, coronary heart disease, asthma, chronic obstructive pulmonary disease, SD=standard deviation. The table is adapted from Strausz et al. (2020) *Sleep apnoea is a risk factor for severe COVID-19.*

The risk for COVID-19 diagnosis in general (N=445) or severe COVID-19 disease, determined as being hospitalised (N=91), was tested for association with OSA. The models were adjusted for age, sex, BMI, hypertension, diabetes, CHD, asthma and COPD. OSA patients had a considerably elevated risk for being hospitalised due to severe COVID-19 (OR=2.93, 95% CI 1.02 to 8.13, P=0.045), (Table 17). OSA did not affect the risk of contracting COVID-19 (P=0.25).

Table 17. Association between obstructive sleep apnoea and severe COVID-19

	OR [95 CI]	**P**
Model 1	3.85 [1.82-8.13]	4.13×10^{-4}
Model 2	3.45 [1.27-9.35]	0.016
Model 3	2.93 [1.02-8.39]	0.045

Model 1 is adjusted for age and sex. Model 2 is adjusted for body mass index (BMI) in addition to the covariates of Model 1. Model 3 is adjusted for BMI, hypertension, diabetes, coronary heart disease, asthma and chronic obstructive pulmonary disease in addition to the covariates of Model 1. OR=odds ratio, CI=confidence interval. The table is adapted from Strausz et al. (2020) *Sleep apnoea is a risk factor for severe COVID-19.*

To investigate the OSA patients diagnosed with COVID-19 in more detail, we assessed the health care data of 305 COVID-19 patients (including eleven patients also diagnosed with OSA) who were treated in Heart and Lung Centre or Department of Oral and Maxillofacial Diseases, HUH, Finland by the end of October 2020. Nine out of eleven had severe OSA and two out of eleven had moderate OSA. Eight out of eleven had treated OSA; seven patients had CPAP therapy and one patient had MAD. Prior to COVID-19 diagnosis, CPAP-treated patients used their appliances 98% of nights with a 6.3 hours mean time used and mean AHI during CPAP treatment was 1.86 events / hour. Despite OSA treatment maintenance, all eleven patients required hospital treatment for either pneumonia or other COVID-19 symptoms, such as high fever. All patients needed non-invasive ventilation support and two required intubation. Mean treatment time in hospital was 15 days. More specific patient characteristics are presented in Table 18.

Table 18. Health care data characteristics of obstructive sleep apnoea individuals diagnosed with COVID-19

N=11	
Age (mean in years, SD)	55.1 (8.0)
Sex (male) (N, %)	9 (81.8)
BMI (mean kg/m^2, SD)	35.08 (5.96)
Diabetes (N, %)	3 (27.3)
Hypertension (N, %)	7 (63.6)
CHD (N, %)	3 (27.3)
CPAP (N, %)	7 (63.6)
MAD (N, %)	1 (9.1)
AHI (mean, SD)	43.3 (20.6)
$ODI_{3\ or\ 4}$ (mean, SD)	39.6 (15.7)
$Sp0_2$ mean (mean, SD)	91.0 (3.3)
$Sp0_2$ min (mean, SD)	79.4 (8.3)
AHI with CPAP (mean, SD)	1.86 (2.07)
ICU (N, %)	3 (27.3)
NIV (N, %)	11 (100)
Intubation (N, %)	2 (18.2)
Treatment time in hospital (mean in days, SD)	15 (8.6)

BMI=body mass index, CHD=coronary heart disease, CPAP=continuous positive airway pressure, MAD=mandibular advancement device, AHI=apnoea-hypopnoea-index, $ODI_{3\ or\ 4}$=oxygen desaturation index; the number of times per hour of sleep that the blood's oxygen level drop 3 or 4 percent from the baseline, SpO_2=oxygen saturation, ICU=intensive care unit, NIV=non-invasive ventilation, SD=standard deviation. The table is adapted from Strausz et al. (2020) *Sleep apnoea is a risk factor for severe COVID-19.*

5.4.2 Meta-analysis

Using our results and the results of previous studies (16-18), a random effect meta-analysis using REML estimation was used to further investigate the role of OSA on COVID-19. This meta-analysis included 15,835 COVID-19 positive individuals with 1,294 OSA patients and showed an over two-fold increase in the risk for hospitalisation for COVID-19 with a previous OSA diagnosis (OR=2.37, 95% CI 1.14 to 4.95, P=0.021). Adjusted estimates were available in the study of Cade et al. 2020 (BMI) and Maas et al. 2020 (BMI, hypertension and diabetes) but not in the study of Cariou et al. 2020 (16-18). The risk became non-significant after adjustment for BMI (OR=1.55, 95% CI 0.88 to 2.72, P=0.13), (Figure 13).

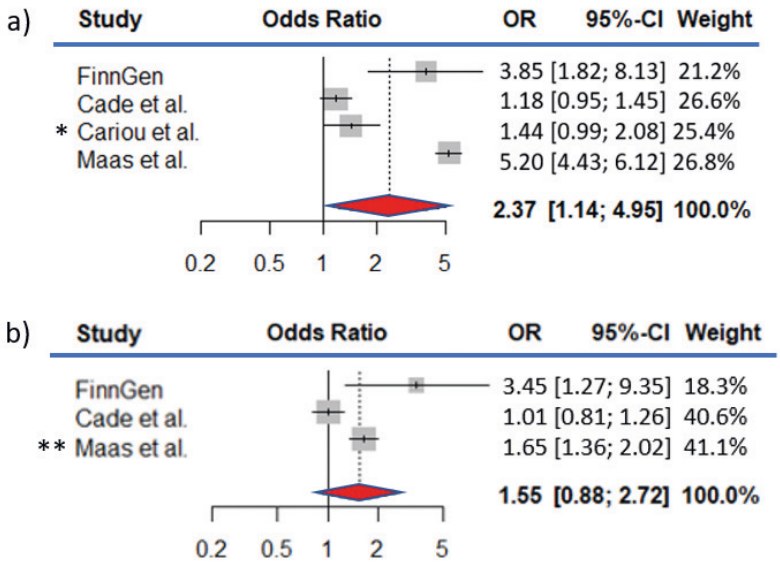

Figure 13. Forest plots of obstructive sleep apnoea and the risk of hospitalisation due to COVID-19. The evidence was combined using random-effect meta-analysis. The data consisted of 15,835 COVID-19 patients including 1,294 patients with a prior OSA diagnosis. A) The model is adjusted for age and sex, and ethnic background if available. *The Cariou et al. study's primary outcome was defined as tracheal intubation and/or death within 7 days of admission. b) The model is adjusted for age, sex, and BMI. ** The Maas et al. study is adjusted for diabetes and hypertension in addition to the aforementioned covariates. The figure is adapted from Strausz et al. (2020) *Sleep apnoea is a risk factor for severe COVID-19.*

6 DISCUSSION

In general, OSA is strongly associated with cardiometabolic health. As OSA research has been so far lacking large genetic studies, we were able to show for the first time a causal relationship between obesity and OSA by MR analysis. This confirms the findings which have been seen previously through epidemiological studies. Additionally, our findings concerning the association between OSA and COVID-19 show that the issues of OSA treatment and diagnosis are particularly topical.

The genetics of OSA have been largely unexplored. Common complex genetic diseases such as OSA do not follow Mendelian patterns of inheritance and characteristically involve many genes that interact with environmental factors. Determining these genes has been challenging prior to the widespread use of the GWAS method. For current analyses, large biobank data sets need to be utilized and combined with phenotype data. In addition, the development of sophisticated statistical methods has allowed cost-effective and accurate analyses of samples and data. The FinnGen project combines these features and has enabled our study of the genetics of OSA.

6.1 Obstructive sleep apnoea diagnosis has excellent validity

OSA diagnosis in the Finnish healthcare context has a validity of over 98% PPV. The interpretation of this finding is that Finnish registries capture OSA patients well and therefore provide a powerful and cost-effective tool to collect and utilize data for research use. Indeed, the studies included in this thesis are based on the fact that the OSA diagnosis is reliable, which provides a comprehensive basis for research. Overall, the registries combined into the FinnGen project have very good validity and coverage (218, 219). These findings further reinforce the evidence of the quality of our data as we study apnoea both genetically and epidemiologically.

6.2 Obstructive sleep apnoea is a risk factor for cardiometabolic outcomes

A large-scale population-based cohort study was utilized that includes up to 25 years of follow-up and over 500,000 person-years. The results are well in line with previous studies concerning OSA as a risk factor for CHD, but differences

also exist. Additionally, women with OSA were at an elevated risk for CHD and T2D which has not been consistently seen in previous studies.

There are several potential explanations for this discrepancy, such as the fact that OSA has traditionally been categorized as a men's disease and less attention has been paid to women when making a diagnosis of OSA. This may have caused power problems in previous analyses. Similarly, disease definitions that define hypopnoeas as ≥4% ODI underestimate AHI in women, who may have more hypopnoeas than obstructions, suggesting that OSA could be more severe in women than polysomnography results show (220).

Among diabetic patients it is increasingly recognized that OSA can accelerate the loss of kidney function, potentially through oxidative stress, hypoxemia, and endothelial dysfunction (98). As this association is also hypothesized to be bidirectional, many studies support the finding that OSA worsens kidney function. As OSA is remarkably common among T2D patients, it should be investigated in individuals who also have T2D in their disease burden (97, 98).

A previous meta-analysis concerning OSA and mortality showed that severe OSA is associated significantly with mortality risk (92). In our study, this risk was increased only among the T2D patient population. One reason for this somewhat different result could be that our OSA patients are mostly well managed, as the diagnoses are derived from secondary health care records and the majority of patients have hence received either CPAP or MAD therapy and conservative treatment (221).

An additional important finding is that OSA with insomnia is more common in women than in men (222). Despite lower AHI, insomniac patients have a higher burden of cardiovascular, pulmonary and psychiatric comorbidities and a lower CPAP adherence compared to patients without insomnia symptoms (223).

6.3 Genetics of obstructive sleep apnoea correlates strongly with cardiovascular and metabolic traits

Biobank data comprised of over 217,000 participants was used to study the genetics behind OSA. A strong genetic predisposition for OSA was shown, identified by five genetic loci. High genetic correlations between OSA and cardiometabolic diseases and other comorbidities were found, with the strongest connections between OSA and BMI, hypertension, T2D and CHD, further supporting earlier epidemiological findings. In addition, the PRS and MR results support a causal role for obesity in OSA.

Several conclusions can be drawn based on these results, which represent the first reported case-control GWAS of OSA. First, genetic variation has a significant contribution to the development of OSA. This is supported by both SNP-based

heritability estimates and the significantly associated loci from the GWAS analysis. Second, the results support the fact that obesity has a central, causal role in OSA. This is supported by the fact that four out of five of our OSA-associated genetic loci were mediated through their associations with BMI. These results are in line with the finding that weight loss is an important contributor to lower AHI. A 10% weight gain increases AHI 32% and correspondingly a 10% decrease in weight has been associated with a 26% decrease in AHI (65). Third, an OSA association near *RMST/NEDD1*, which was specific for OSA independent of BMI, was identified. The lead SNP could not be replicated in independent cohorts, but it was associated with antidepressant purchases, suggesting a connection between this locus and the regulation of sleep and mood. This finding may also reflect the earlier observation that depression is prevalent among patients with OSA (100). Fourth, a strong genetic correlation was observed between OSA and sleep traits, especially with sleepiness and sleep efficiency. This finding highlights the pathological effects of OSA on sleep.

As OSA and its associated diseases seem to share a common genetic basis, both CPAP and MAD treatments have numerous positive health effects not only for OSA but also for its comorbidities through e.g., lowering blood pressure and improving sleep-related symptoms as well as the quality of life, creating a *virtuous cycle*. Therefore, our genetic correlations also emphasize the importance of treating OSA.

6.4 Obstructive sleep apnoea is a risk factor for severe COVID-19

The role of OSA as a risk factor for COVID-19 leading to hospitalisation was studied, revealing a higher risk for a hospitalisation in patients with moderate or severe OSA, independent of BMI and other known risk factors for OSA or severe COVID-19. This finding is comparable to the risk among individuals with diabetes, where an elevated risk has been previously reported for severe COVID-19 (224).

There are several potential explanations for how OSA may predispose to severe COVID-19. Individuals with OSA often have one or more comorbidities that are known risk factors for severe COVID-19. For example, high BMI increases the risk for severe COVID-19. Additionally, OSA may exacerbate the symptoms of severe COVID-19 as decreased oxygen saturation levels occur in OSA during sleep (5).

Treatment information concerning OSA patients who had contracted COVID-19 was also collected. This revealed the fact that despite good management of OSA, patients with moderate or severe OSA were more likely to develop a

severe form of COVID-19. All OSA patients required hospital care, suggesting that moderate or severe OSA is a risk factor even if well treated.

Based on our results and the results of previous studies (16-18), a meta-analysis was carried out to increase the knowledge of the role of OSA on COVID-19 hospitalisation. Our meta-analysis suggested OSA is a risk factor and showed an association of over 2-fold increased risk related to COVID-19 hospitalisation, but after BMI-adjustment the result of the meta-analysis became non-significant. This suggests confirmation of the known fact that obese individuals have an increased risk for the severe form of COVID-19.

The findings based on our report are in line with previous studies on COVID-19 comorbidities and OSA, regardless of slightly different endpoint definitions (contracting COVID-19, severity of the disease, mechanical ventilation, and death). Concordantly all studies showed a significant association with COVID-19 severity and OSA (16-18) but differences also existed. Only one study showed a statistically significant association between OSA and severe COVID-19 after adjusting for BMI (18). These findings indicate that while OSA is an expected a risk factor for severe COVID-19 disease, evaluating the magnitude of this association would benefit from harmonized analyses across different cohorts where comorbidities and disease endpoints are similarly assessed.

Our findings, together with earlier reports, suggest that OSA should be taken into account when assessing the risk of developing life-threatening complications of COVID-19, paying special attention to individuals with moderate or severe OSA.

6.5 Strengths

Accurately-defined OSA diagnoses are the core of the registry-based studies in this thesis. Here we are able to show that the diagnosis has excellent validity, which reinforces the utilization of the diagnosis derived from Hospital Discharge and Causes of death registries in these studies. The main strength of this work is the large and comprehensively collected data that also include a long follow-up, reaching up to 25 years and including over 500,000 person-years. The FinnGen project combines registry data with genotype data and thus creates a unique environment to study common complex diseases, such as OSA.

6.6 Limitations

The studies introduced in this thesis do have some limitations. First, registry-based ascertainment through hospitalisation may miss non-hospitalised cases (false negatives) and treatment information such as CPAP compliance or MAD usage is

missing from the data. The validation study was performed using data from only one hospital district, which does not guarantee that the data is as consistent in other parts of the country. However, HUS is the largest hospital district and the majority of the patients in the study were from that area. Second, in Study II, due to a relatively small number of cases in the replication datasets, our statistical power was limited in the replication analysis. Additionally, the finding of rs185932673 should be interpreted cautiously as the variant is rare in the Finnish population and the association was not replicated in the other study samples. In Study III, a relatively small number of individuals have had COVID-19 during the first wave of the disease in Finland. Therefore, because of the relatively small sample size, the confidence intervals are relatively large.

6.7 Impact and generalizability

The epidemiology of OSA has been extensively studied and its effects on health and related comorbidities are fairly well known. However, the genetics of OSA has been largely unstudied. Here we present results utilizing nationwide registries and biobank data to show that genetic variation plays an important role in the development of OSA, and this association is primarily mediated through BMI. In addition, OSA is both epidemiologically and genetically associated with its comorbidities, indicating that OSA is a heterogenic disease with several distinct comorbidities. This would be beneficial to consider when treating patients with OSA.

During this exceptional pandemic time, we have created more knowledge concerning OSA as a risk factor for severe COVID-19. Therefore, in the assessment of patients with a suspected or confirmed COVID-19 diagnosis, OSA should be recognised as one of the risk factors for developing a severe form of the disease.

6.8 Future research

As OSA is a very common disease including many, even life-threatening, adverse effects, attention should be paid not only to treatment guidance and identification of OSA and its comorbidities, but also to prevention. In addition, large datasets with genetic information may allow identification of even more accurate disease patterns, creating opportunities to study the genetics underlying craniofacial traits. These features provide information concerning an individual's genetic OSA risk and possibly individualized prevention strategies and treatments.

7 CONCLUSIONS

I. After adjusting for conventional cardiovascular disease risk factors, obstructive sleep apnoea (OSA) was a risk factor for coronary heart disease (CHD), type 2 diabetes (T2D) and notably for diabetic kidney disease (DKD). OSA also increased the risk for mortality among the T2D population. The association between OSA, CHD and T2D risk was also elevated in women, who have traditionally received less attention in the diagnosis and treatment of OSA than men. These findings emphasize the need to search for signs of OSA also in women, and in patients with T2D.

II. Our findings highlight the causal links between obesity and OSA but also provide possible evidence for non-body mass index (BMI)-dependent genetic effects. In addition to BMI, we show that genetic effects that modify the risk of cardiometabolic diseases, depression, hypothyroidism, asthma and inflammatory rheumatic diseases (IRD) are also correlated with the genetic effects for OSA. This shows that the observed comorbidities between OSA and these diseases may have a joint genetic basis.

III. Our results support the fact that OSA is a heterogenic disease with several distinct comorbidities, which would be beneficial to consider when treating patients with OSA.

IV. Patients with moderate or severe OSA had 2.93 times higher risk of hospitalisation when diagnosed with COVID-19 than non-OSA individuals. Our findings may suggest that, in the assessment of patients with suspected or confirmed COVID-19, OSA should be recognized as one of the risk factors for developing a severe form of the disease.

ACKNOWLEDGEMENTS

This doctoral thesis is the outcome of a fluent and open collaboration between the experts of different fields and was carried out under the Doctoral Programme in Population Health, University of Helsinki, in the Department of Oral and Maxillofacial Diseases, Helsinki University Hospital and in the Institute for Molecular Medicine Finland, FIMM, University of Helsinki, hand in hand with my specialisation studies in orthodontics. The work was funded by grants from the Finnish Dental Association Apollonia and Suomen Naishammaslääkärit and this contribution is gratefully acknowledged.

I want to thank my supervisors Associate Professor Tuula Palotie and Professor Samuli Ripatti for guiding me through this project. I'm highly privileged that I have had an opportunity to work with the top experts and I could have not asked for a better team. This journey has also provided me with opportunities that I did not know even existed.

Dear Tuula, I'm deeply grateful for you not only because you have been my doctoral thesis and clinical studies supervisor, but also that you saw me as a potential candidate for this project by introducing me to this research field by giving me an opportunity to work with this very interesting project. That meant a lot to me.

Dear Samuli, I'm very honoured that you offered me a chance to work in your group and learn how the top scientists in the genetics field work. I highly admire your vast knowledge of the scientific fields and forever encouraging advice during these years. Thank you also for accepting my request to serve as the custos at my defence.

Thank you Adjunct Professor Ulla Anttalainen and Professor Teemu Niiranen for reviewing this thesis. I highly appreciate your valuable and wise comments and suggestions which improved this work significantly. I warmly thank Professor Marju Orho-Melander for accepting the request to serve as my opponent.

I would like to thank the members of my follow-up group, Associate Professor Sirpa Kivirikko and Professor Pirkko Brander for your guidance during our discussions.

There are many co-workers without whom this thesis would have never happened. I especially want to thank Professor Aarno Palotie. Thank you for your wisdom, advice and help throughout this project. Your constant passion for science is very inspiring. Thank you Sanni Ruotsalainen for your contribution and time especially concerning the statistical analyses beginning from the very early stages of this project. I enjoyed our many discussions about science and not-so-scientific topics. Thank you Docent Aki Havulinna for the statistical support and

patience when answering my endless questions concerning data and analyses. I'm very grateful that I met Hanna M. Ollila while working with this project. She has always been willing to give her fresh knowledge and most of all, I am thankful for her mentoring-like support and friendship. Without you making science would not have been so much fun. Juha Karjalainen, I have been privileged to receive your help always when needed. Nina Mars, it's been a pleasure to work with such a talented young researcher as you are. Tuomo Kiiskinen, thank you for your help and great advice. Thank you Elina Luonsi for your contribution with the data collection. Thank you Elina Kilpeläinen, Mervi Kinnunen and Mervi Aavikko for general assistance. Thank you Paula Bergman, Heidi Hautakangas, Nella Junna, Sini Kerminen, Jukka Koskela, Mitja Kurki, Juulia Partanen and Mary Pat Reeve for creating a great atmosphere to study and all of the help which I have gotten during these years. I would like to acknowledge Sari Kivikko. You have always found the way to solve tricky time-table puzzles and other practical things. Thank you Lea Urpa for your great contribution concerning the language check of this work.

My deepest gratitude goes to our Sleep apnea -group members who have participated in this project for all these years. Docent Adel Bachour, thank you for providing your enormous knowledge concerning sleep apnoea and constant kindness during this project. Thank you Associate Professor Tiinamaija Tuomi and Professor Leif Groop for providing your own expertise for this work. Thank you Professor Veikko Salomaa for offering your wide knowledge of science. I would also like to thank Professor Antti Mäkitie for his participation by offering his expertise. I am deeply grateful for all the knowledge I have gained from all of you during these years.

Thank you Docent Pekka Nieminen for providing me with a great basis of genetics when I had the opportunity to work in your lab. My gratitude goes also to Professor David Rice who gave me valuable education during my specialisation studies through many great discussions during our journal clubs and encouraging me think critically. I want also to acknowledge my orthodontic colleagues who I befriended during my specialisation for many enjoyable moments.

I would also like to thank my dear friends Laura, Jetta and Pauliina. I'm grateful to have you in my life!

My deepest gratitude goes to my mother, Eine, who has constantly been there for us and helped out with my family by babysitting our small children. I also have warm memories of my late father Juhani, who gave me inspiration into research through following his scientific career in childhood and throughout my life. Thank you Tapani, Kati, Toni and Tiia for your refreshing company.

Most of all, I want to express my deepest and greatest gratitude to my husband Tommi, who has stood with me all these years. Thank you for your endless support and trust during our adventures. I love you. Especially I'm grateful for our children, Samuel and Joona, you cute little treasures. Thank you for showing me what pure love and happiness is.

Satu Strausz
Helsinki 2021

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